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(54) ATP binding cassette transporter 1 (ABC1) gene polymorphisms and uses thereof for the diagnosis and treatment of lipid disorders, cardiovascular diseases and inflammatory diseases

(57) The present invention provides four common polymorphisms in the gene encoding the ATP-binding cassette transporter-1 (*ABC1*), which are associated with decreased ApoA-I mediated efflux of cholesterol, the first step of reverse cholesterol transport. The provided data can be taken as evidence that common polymorphisms in *ABC1* directly affect cellular lipid homeostasis, which is a key factor in the atherogenetic process. The frequencies of three of the common *ABC1* variants are significantly increased in a population of men having low HDL-C levels and established CHD relative to CHD-free control subjects. The use of the provided ABC1 polymorphisms for the diagnosis and treatment of lipid disorders, cardiovascular diseases, and inflammatory diseases like psoriasis and lupus erythematodes is claimed.

Figure 2: human *ABC1* gene – protein translation

```
   AAACCCCGTAATTGCGAGCGAGAGTGAGTGGGGCCGGGACCCGCAGAGCC
 1 ---------+---------+---------+---------+---------+ 50

   GAGCCGACCCTTCTCTCCCGGGCTGCGGCAGGGCAGGGCGGGGAGCTCCG
51 ---------+---------+---------+---------+---------+ 100

   CGCACCAACAGAGCCGGTTCTCAGGGCGCTTTGCTCCTTGTTTTTTCCCC
101 ---------+---------+---------+---------+---------+ 150

   GGTTCTGTTTTCTCCCCTTCTCCGGAAGGCTTGTCAAGGGGTAGGAGAAA
151 ---------+---------+---------+---------+---------+ 200

   GAGACGCAAACACAAAAGTGGAAAACAGTTAATGACCAGCCACGGCGTCC
201 ---------+---------+---------+---------+---------+ 250

   CTGCTGTGAGCTCTGGCCGCTGCCTTCCAGGGCTCCCGAGCCACACGCTG
251 ---------+---------+---------+---------+---------+ 300

   GCCGTGCTGGCTGAGGGAACATGGCTTGTTGGCCTCAGCTGAGGTTGCTG
301 ---------+---------+---------+---------+---------+ 350
                      M  A  C  W  P  Q  L  R  L  L   -

   CTGTGGAAGAACCTCACTTTCAGAAGAAGAcaaacatgtcagctgttact
351 ---------+---------+---------+---------+---------+ 400
     L  W  K  N  L  T  F  R  R  R  Q  T  C  Q  L  L  L   -

   ggaagtggcctggcctctatttatcttcctgatcctgatctctgttcggc
401 ---------+---------+---------+---------+---------+ 450
     E  V  A  W  P  L  F  I  F  L  I  L  I  S  V  R  L   -

   tgagctacccaccctatgaacaacatgaatgccattttccaaataaagcc
451 ---------+---------+---------+---------+---------+ 500
       S  Y  P  P  Y  E  Q  H  E  C  H  F  P  N  K  A   -

   atgccctctgcaggaacacttccttgggttcaggggattatctgtaatgc
501 ---------+---------+---------+---------+---------+ 550
     M  P  S  A  G  T  L  P  W  V  Q  G  I  I  C  N  A   -

   caacaaccccctgtttccgttacccgactcctggggaggctcccggagttg
551 ---------+---------+---------+---------+---------+ 600
     N  N  P  C  F  R  Y  P  T  P  G  E  A  P  G  V  V   -
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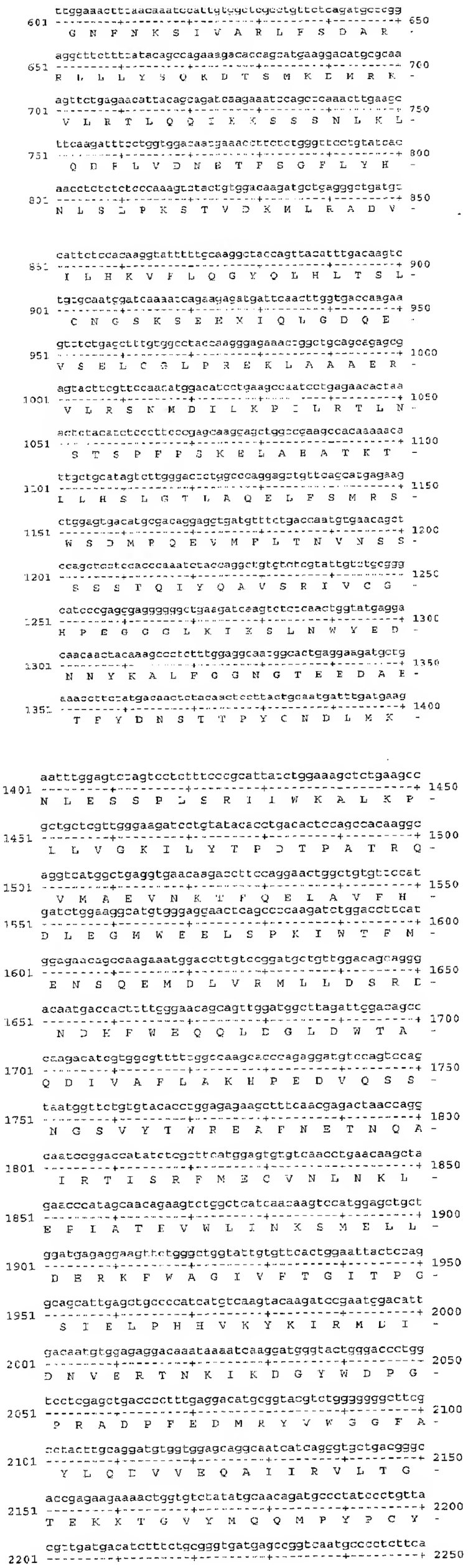

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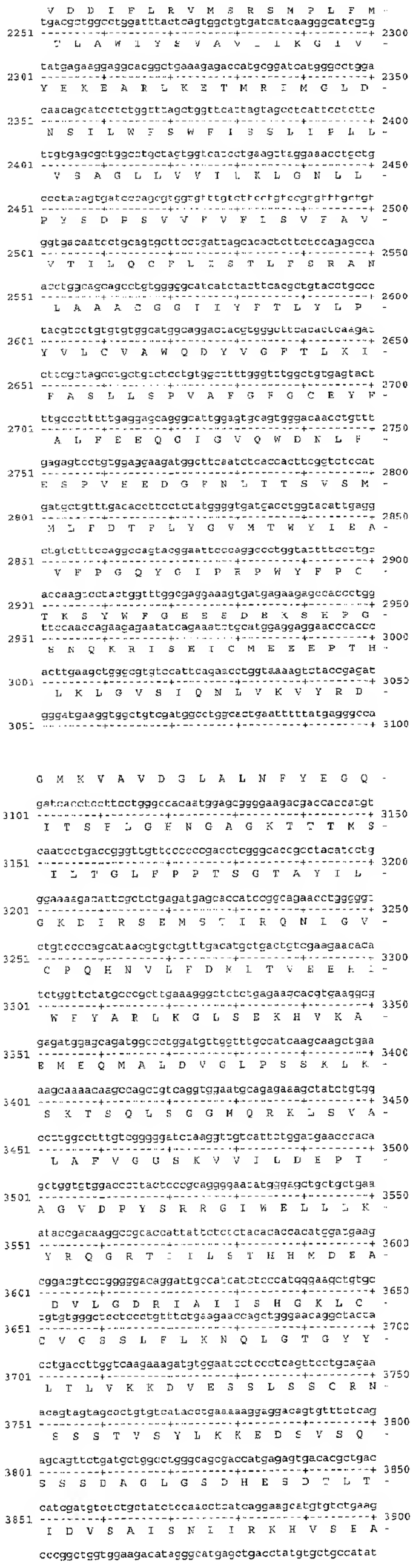

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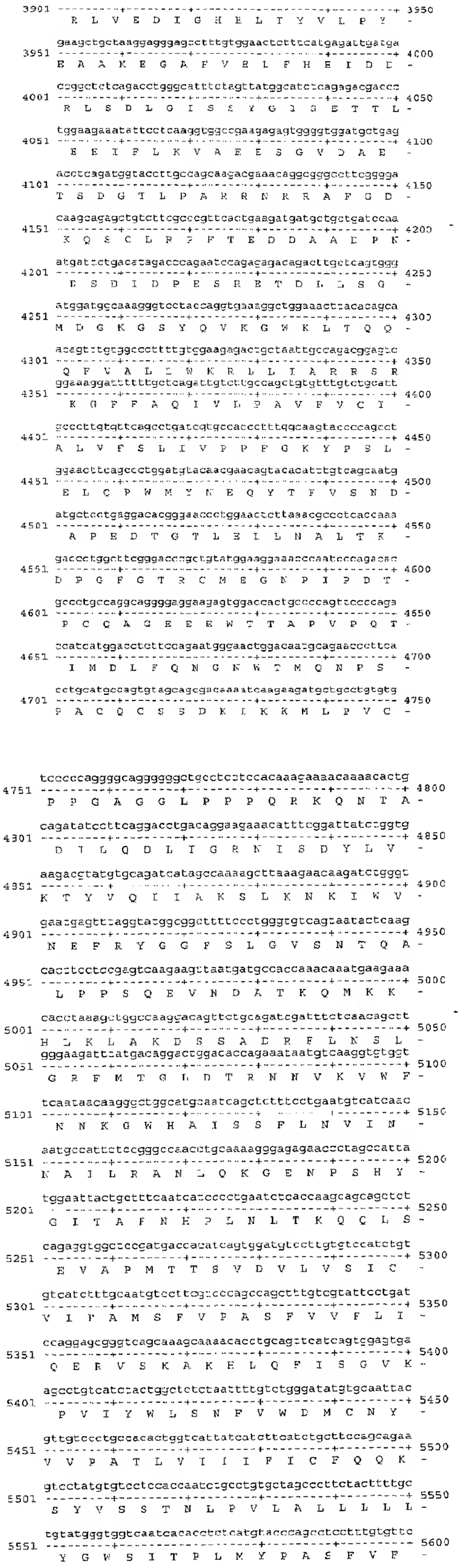

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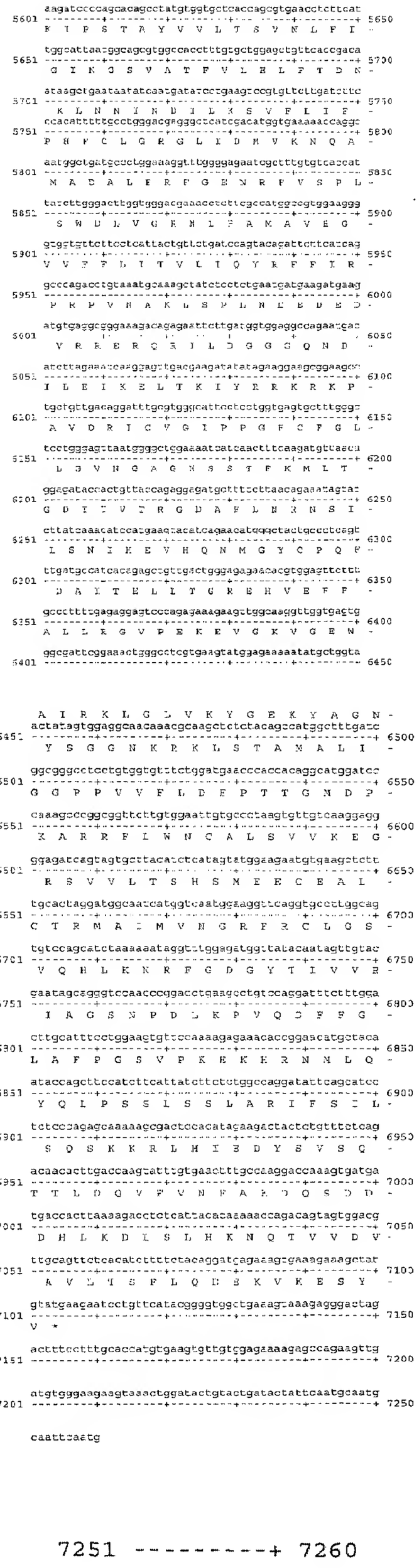

Description

Background of the invention

[0001] Epidemiological studies have established that plasma high density lipoprotein cholesterol (HDL-C) concentrations are inversely associated with the incidence of coronary heart disease (CHD) (1-4). Moreover, HDL deficiency is the most common lipid abnormality observed in patients with premature CHD (5,6), with nearly half of all CHD patients having low concentrations of HDL-C. Genetic factors account for approximately 50% of the variation in HDL-C levels in the general population (7), with several common mutations identified in genes encoding for proteins involved in the regulation of plasma HDL-C concentrations and, ultimately, playing a role in the development of CHD (8).

[0002] Recently, we (9,10) and others (11-15) demonstrated that familial HDL deficiency (FDH) syndromes with either splenomegalie or atherosclerosis (e.g. Tangier disease (TD); familial hypoalphaglycoproteinaemia) are rare HDL-deficiencies caused by mutations in the gene encoding the ATP-binding cassette transporter-1 (ABC1). ABC1 is a member of the ATP binding cassette family of proteins that are involved in the transmembrane transport of a variety of different molecules, including bile acids, steroid hormones, ions, amino acids, sugars and vitamins (16-19). TD is characterized by severely diminished plasma HDL-C concentrations, reduced levels of low density lipoprotein cholesterol (LDL-C), the deposition of cholesteryl esters in the reticulo-endothelial system with splenomegaly and enlargement of tonsils and lymph nodes (20), as well as a predisposition to neuropathy and CHD (21,22). The HDL deficiency in these patients has been related to a rapid catabolism of HDL and a decrease in ApoA-I induced lipid efflux from various cells including mononuclear phagocytes (23-25). Familial hypoalphaglycoproteinaemia represents the other clinical phenotype that is characterized by a similar disturbance in ApoA-I metabolism but is associated with an enhanced risk of CHD rather than splenomegalie. ApoA-I mediated cellular cholesterol efflux is the first step in the reverse cholesterol transport and prevents excessive cholesterol accumulation in peripheral cells. In addition, this process is suggested to contribute to the formation of mature HDL particles, thereby influencing the pool size of HDL in the plasma.

[0003] Although mutations in *ABC1* cause TD, the precise molecular mechanism by which they do so remains unknown. Also, little is known about the association of ABC1 and HDL metabolism in individuals not affected with TD. Previous investigations on the function of ABC1 in mice have revealed its role in the engulfment of apoptotic cells by macrophages (26) and in macrophage interleukin-1β secretion (27). We have further shown that ABC1 is a cholesterol-responsive modulator of cellular lipid efflux (10) and, thus, plays a significant role in cholesterol homeostasis. Moreover, the ABC1-knockout mice (ABC1-/-) show massively reduced levels of serum lipids and lipoproteins. The expression of ABC1 in mucosa cells of the small intestine and the altered lipoprotein metabolism in ABC1-/- mice allows the conclusion that ABC1 plays also a major role in intestinal resorption and translocation of lipids into the lymph-system.

Summary of the invention

[0004] Four different polymorphisms in *ABC1* (G596A, T1136C, A2589G, and G3456C) were identified in different Tangier kindreds as well as 516 healthy control subjects. Each of these polymorphisms affect the primary structure of the gene product.

[0005] A frequent coincidence of the G596A and A2589G polymorphisms was identified indicating that those two mutations are in linkage disequilibrium.

[0006] The two most common polymorphisms (G596A and A2589G) are both associated with a decreased in vitro ApoA-I mediated efflux of cholesterol from mononuclear phagocytes emphasizing the role of ABC1 in lipid efflux, the first step in reverse cholesterol transport. These results also are evidence that common polymorphisms in *ABC1* directly affect cellular lipid homeostasis, which is a key factor in the atherogenic process. A reduction of ApoA-I mediated efflux of cholesterol from mononuclear phagocytes is also a typical feature of Tangier disease.

[0007] Plasma lipoproteins, and HDL-cholesterol in particular, are not affected, probably because the effect of the *ABC1* polymorphisms on the protein function is compensated by other factors controlling plasma HDL levels.

[0008] Three common variants in the gene encoding ABC1 (G596A, A2589G, and G3456C) occur with significantly increased frequency in a population of men having low HDL-C as their primary lipid abnormality and established CHD, providing evidence for a role of *ABC1* in the premature CHD associated with common HDL deficiency states.

[0009] In summary, the identified polymorphisms in the ABC1 gene can be used for diagnosis and therapy of lipid disorders, cardiovascular diseases, and inflammatory diseases.

Abbreviations

[0010]

ABC	ATP-binding cassette
ANC	Anchor
ApoA	Apolipoprotein A
ApoB	Apolipoprotein B
ApoE	Apolipoprotein E
ATP	Adenosine triphosphate
BMI	Body mass index
bp	Base pairs
cAMP	Cyclic adenosin monophosphat
cDNA	Copy DNA
CH	Cholesterol
CHD	Coronary heart disease
DET	Detection
DPM	Disintegrations per minute
E-LDL	Enzymatically modified LDL
f	Forward
Fluo	Fluorescein
FOS	Framingham Offspring Study
HDL	High density lipoprotein
HDL-C	HDL-cholesterol
kDa	Kilo Dalton
LDL	Low density lipoprotein
LDL-C	LDL-cholesterol
M-CSF	Macrophage colony stimulating factor
ph	Phosphorylation
r	Reverse
SD	Standard seviation
SDS	Sodium dodecyl sulfate
TD	Tangier Disease
TG	Triglycerides
VA-HIT	Veterans Affairs Cooperative HDL Cholesterol Intervention Trial
wt	Wild type

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[0011]

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Description of Tables:

Table 1:

[0012] Summary of the characteristics of the study population consisting of 516 healthy blood donors. Data are expressed as mean ± SD. n indicates the number of analyzed individuals.

Table 2:

[0013] Frequency of *ABC1* polymorphisms G596A, A2589G, G3456C, and T1136C in healthy control subjects. Genotyping was performed by LightCycler (Roche Diagnostics) PCR analysis. Data provide the relative percentage of homozygous, heterozygous or wild type individuals at the different polymorphic sites. n indicates the number of analyzed individuals.

Table 3:

[0014] Distribution of G596A and A2589G polymorphisms in healthy control subjects. Data represent absolute numbers of subjects with the respective allele combination. Data in parenthesis provide the relative percentage within each group.

Table 4:

[0015] Amplification primers and hybridization probes for the LightCycler polymorphism analysis. Hybridization probes are designated as detection (DET) or anchor (ANC) probes. As indicated, they are labeled with LightCycler-Red 640 or 705 and fluorescein, respectively. Phosphorylation (ph) of the 3'-end prevents the elongation of the detection probe. All detection probes recognize wild-type sequences, with the exception of the probe DET1136/mut. Fluo - fluorescein.

Table 5:

[0016] Distribution of lipid and lipoprotein values in healthy individuals with different *ABC1* polymorphisms. HDL-C and ApoA-I levels in homozygous, heterozygous, and wild type male and female individuals are presented. Data are expressed as mean ± SD. n indicates the number of analyzed individuals.

Table 6:

[0017] Cholesterol and phopholipid efflux from mononuclear phagocytes isolated form healthy individuals homozygous for the wild type nucleotide at all four polymporphic sites and from healthy individuals ho-

mozygous for - the polymorphic allele at position 596 or 2589. Lipid efflux is expressed as percent of basal unstimulated efflux observed in serum-free macrophage medium without ApoA-I. Data are the mean ± SD of the indicated number (n) of different subjects. Efflux analysis for each subject was performed at least in quadruplicate. P values were calculated by independent Student T-test for wt/wt versus G596A and A2589G, respectively.

Table 7:

[0018] Characteristics of *FOS* and VA-HIT subjects. Results are listed as means±SD. *Significantly different from male FOS control subjects, P<0.01.

Table 8:

[0019] Genotype distribution of the *ABC1* polymorphisms in FOS versus VA-HIT subjects. Values in parentheses are expressed as a percentage of the total number of subjects within each group.

Table 9:

[0020] Association of the G596A and A2589G *ABC1* polymorphisms in FOS and VA-HIT subjects. Data represent absolute numbers of subjects out of a total of 1,008 for FOS and 1,014 for VA-HIT, whereas values in parentheses (%) provide the relative percentage of subjects within each group (FOS or VA-HIT). Significant differences in proportions between FOS and VA-HIT are presented in bold face type.

Table 10:

[0021] Association of the G596A and G3456C *ABC1* polymorphisms in FOS and VA-HIT subjects. Data represent absolute numbers of subjects out of a total of 1,006 for FOS and 1,014 for VA-HIT, whereas values in parentheses (%) provide the relative percentage of subjects within each group (FOS or VA-HIT). Significant differences in proportions between FOS and VA-HIT are presented in bold face type.

Table 11:

[0022] Association of the A2589G and G3456C *ABC1* polymorphisms in FOS and VA-HIT subjects. Data represent absolute numbers of subjects out of a total of 1,008 for FOS and 1,014 for VA-HIT, whereas values in parentheses (%) provide the relative percentage of subjects within each group (FOS or VA-HIT). Significant differences in proportions between FOS and VA-HIT are presented in bold face type.

Table 1:

Summary of the characteristics of the study population consisting of 516 healthy blood donors.						
	n	Age	Cholesterol mmol/l	Triglycerides mmol/l	HDL-C mmol/l	LDL-C mmol/l
Men	274	27.3 ± 7.7	4.95 ± 1.13	1.23 ± 0.85	1.43 ± 0.35	2.97 ± 1.01
Women	242	30.5 ± 10.2	5.63 ± 1.10	1.19 ± 0.56	1.72 ±0.45	3.24 ± 1.02

Table 2:

Frequency of ABC1 Polymorphisms in healthy control subjects				
	n	**Homozygous**	**Heterozygous**	**Wild Type**
G596A	511	8.0 %	43.5 %	48.5 %
A2589G	514	2.3 %	22.8 %	74.9 %
G3456C	516	0.0 %	5.0 %	95.0 %
T1136C	515	0.0 %	0.6 %	99.4 %

Table 3:

Distribution of G596A and A2589G Polymorphisms in healthy control subjects			
	G596A		
	596A/A	596G/A	596G/G
A2589G			
2589G/G	4 (9.8%)	6 (2.7%)	2 (0.8%)
2589A/G	14 (34.1%)	61 (27.6%)	39 (15.7%)
2589A/A	23 (56.1%)	154 (69.7%)	207 (83.5%)

Table 4:

Amplification primers and hybridization probes for the LightCycler polymorphism analysis	
G596A	
DET596/705	5'-LC Red 705-GCCTACCAAGGGAGAAACTG-ph
ANC596	5'-CTTGGTGACCAAGAAGTTTCTGAGCTTTG-Fluo
FLA596-f	5'-TTTTGCAAGGCTACCAGTTACA
FLA596-r	5'-CAGGATTGGCTTCAGGATGT
T1136C	
DET1136/mut	5'-LC Red 640-ACTCCAGGCGAACAAGACC-ph
ANC1136/B	5'-TTTCTGCGGTCCCTGGCTCCCCACC-Fluo
FLA1136-f	5'-AAGTCTGATGCAGACCAGAGC
FLA1136-r	5'-CGGACAAGGTCCATTTCTTG
A2589G	
DET2589rev	5'-LC Red 640-CTTTCTGATATTCTCTTCTGG-ph
ANC2589rev	5'-TTAAAGAAAGAGCAGGAGGTCAACAGCACT-Fluo
FLA2589-f	5'-AGGCCCTGGTATTTTCCTTG
FLA2589-r	5'-CCCTGGAGTGGTTTCACAGT
G3456C	
DET3456rev	5'-LC Red 640-CGTGTCACTCTCATGGTCG-ph
ANC3456rev	5'-AGAAACCCCAGAGTCCTTACCGATGGTC-Fluo
FLA3456-f	5'-GGACAGTGTTTCTCAGAGCAG
FLA3456-r	5'-AGCAGCAAACCTTGAGTCAG

Table 5:

Distribution of lipid and lipoprotein values in healthy individuals with different *ABC1* polymorphisms			
	Homozygous	Heterozygous	Wild Type
G596A	*596A/A*	*596G/A*	*596G/G*
men	n=19	n=127	n=126
HDL-C (mmol/l)	1.41 ± 0.26	1.45 ± 0.36	1.41 ± 0.34
ApoA-I(g/l)	1.51 ± 1.6	1.49 ± 2.5	1.48 ± 2.3
women	n=22	n=95	n=122
HDL-C (mmol/l)	1.82 ± 0.45	1:75 ± 0.46	1.73 ± 0.44
ApoA-I(g/l)	1.77 ± 3.9	1.79 ± 3.6	1.76 ± 3.3
A2589G	*2589G/G*	*2589A/G*	*2589A/A*
men	n=7	n=54	n=212

Table 5: (continued)

Distribution of lipid and lipoprotein values in healthy individuals with different *ABC1* polymorphisms			
	Homozygous	Heterozygous	Wild Type
A2589G	*2589G/G*	*2589A/G*	*2589A/A*
HDL-C (mmol/l)	1.50 ± 0.28	1.39 ± 0.32	1.44 ± 0.36
ApoA-I(g/l)	1.62 ± 21	1.47 ± 24	1.49 ± 24
women	n=5	n=63	n=173
HDL-C (mmol/l)	1.94 ± 0.40	1.80 ± 0.37	1.72 ± 0.48
ApoA-I(g/l)	2.02 ± 28	1.79 ± 32	1.76 ± 36
G3456C	*3456C/C*	*3456G/C*	*3456G/G*
men	n=0	n=15	n=228
HDL-C (mmol/l)		1.37 ± 0.27	1.44 ± 0.35
ApoA-I(g/l)		1.50 ± 26	1.49 ± 24
women	n=0	n=11	n=262
HDL-C (mmol/l)		1.84 ± 0.46	1.74 ± 0.45
ApoA-I (g/l)		1.79 ± 34	1.77 ± 35

Table 6:

Cholesterol and phopholipid efflux from mononuclear phagocytes isolated form healthy individuals homozygous for the wild type nucleotide at all four polymporphic sites and from healthy individuals homozygous for the polymorphic allele at position 596 or 2589.			
A. Cholesterol efflux: ApoA-I [10 μg/ml]			
	wt/wt 596G/G; 2589A/A	**596A/A**	**2589G/G**
all probands	128.7 ± 17.5% (n=18)	97.7 ± 22.4% (n=8; p<0.001)	103.4 ± 18.7% (n=11; p<0.001)
men	126.7 ± 16.6% (n=14)	98.8 ± 43.8% (n=2; n.s.)	104.5 ± 23.0% (n=6; p<0.025)
women	135.6 ± 21.4% (n=4)	97.3 ± 18.0% (n=6; p<0.001)	102.0 ± 14.3% (n=5; p<0.025)
B. Cholesterol efflux: ApoA-I [10 μg/ml]+ 24 h pretreatment with forskolin [10 μM]			
	wt/wt 596G/G; 2589A/A	**596A/A**	**2589G/G**
men and women	121.8 ± 16.4% (n=12)	126.7 ± 37.4% (n=8; n.s.)	121,5 ± 38.8% (n=10; n.s.)
C. Phospholipid efflux: ApoA-I [10 μg/ml]			
	wt/wt 596G/G; 2589A/A	**596A/A**	**2589G/G**
men and women	125.9 ± 15.3% (n=18)	117.3 ± 27.1% (n=8; n.s.)	114,9 ± 32.6% (n=10; n.s.)
D. Phospholipid efflux: ApoA-I [10 μg/ml]+ 24 h pretreatment with forskolin [10 μM]			
	wt/wt 596G/G; 2589A/A	**596A/A**	**2589G/G**
men and women	135.4 ± 20.2% (n=12)	136.7 ± 12.8% (n=8; n.s.)	124.5 ± 25.8% (n=10; n.s.)

Table 7:

Characteristics of FOS and VA-HIT subjects		
	FOS	**VA-HIT**
N	1014	1014
Age (years)	53±9	64±7*
BMI (kg/m^2)	27.7±3.9	29±5*
TC (mg/dL)	206±36	175±25*
LDL-C (mg/dL)	136±33	111±23*
HDL-C (mg/dL)	44±12	32±5*
TG (mg/dL)	138±101	161±68
TC:HDL-C	5.1±1.6	5.7±1.1*

*Significantly different from male FOS control subjects, P<0.01.

Table 8:

Genotype distribution of the *ABC1* polymorphisms in FOS versus VA-HIT subjects			
Mutation	**FOS**	**VA-HIT**	**P**
G596A			
Total n	1013	1014	
GG (%)*	543 (53.6)	454 (44.8)	
GA (%)	402 (39.7)	484 (47.7)	
AA (%)	68 (6.7)	76 (7.5)	
A Allele frequency	**0.266**	**0.314**	**0.000**
A2589G			
Total n	1014	1014	
AA (%)	779 (76.8)	707 (69.7)	
AG (%)	222 (21.9)	285 (28.1)	
GG (%)	13 (1.3)	22 (2.2)	
G Allele frequency	0.122	0.162	0.001
G3456C			
Total *n*	1013	1014	
GG (%)	965 (95.3)	940 (92.7)	
GC (%)	43 (4.2)	70 (6.9)	
CC (%)	5 (0.5)	4 (0.4)	
C Allele frequency	0.026	0.038	0.032

Table 9:

Association of the G596A and A2589G *ABC1* polymorphisms in FOS and VA-HIT subjects						
			G596A			
	GG		**GA**		**AA**	
	FOS	**VA-HIT**	FOS	VA-HIT	FOS	VA-HIT
A2589G						
AA	459	360	77	89	5	5
	(45.6)	**(35.5)**	(7.6)	(8.8)	(0.5)	(0.5)
AG	268	30	125	166	5	12
	(26.6)	**(30.2)**	**(12.4)**	**(16.4)**	(0.5)	(1.2)
GG	47	41	18	30	3	5
	(4.7)	(4.0)	**(1.8)**	**(3.0)**	(0.3)	(0.5)

Table 10:

Association of the G596A and G3456C *ABC1* polymorphisms in FOS and VA-HIT subjects						
G596A						
	GG		**GA**		**AA**	
	FOS	VA-HIT	FOS	VA-HIT	FOS	VA-HIT
G3456C						
GG	508	426	27	26	3	2
	(50.5)	(42.0)	(2.7)	(2.6)	(0.3)	(0.2)
GC	384	450	14	34	2	0
	(38.2)	**(44.4)**	**(1.4)**	**(3.4)**	(0.2)	(0)
CC	66	64	2	10	0	2
	(6.6)	(6.3)	(0.2)	(1.0)	(0)	(0.2)

Table 11:

Association of the A2589G and G3456C *ABC1* polymorphisms in FOS and VA-HIT subjects						
A2589G						
	AA		**AG**		**GG**	
G3456C						
GG	746	666	204	257	11	17
	(74.0)	**(65.7)**	**(20.2)**	**(25.3)**	(1.1)	(1.7)
GC	26	39	15	27	1	4
	(2.6)	(3.8)	**(1.5)**	**(2.7)**	(0.1)	(0.4)
CC	3	2	2	1	0	1
	(0.3)	(0.2)	(0.2)	(0.1)	(0)	(0.1)

Description of specific embodiments

Identification of four polymorphisms in the gene encoding the human ATP-binding cassette transporter 1 (*ABC1*) and association of these polymorphisms with decreased ApoA-I mediated efflux of cholesterol

[0023] This invention provides the four polymorphisms G596A, T1136C, A2589G, and G3456C in the *ABC1* gene (*ABC1* gene see Figures 1 and 2), which affect the primary structure of the gene product. The frequencies of these *ABC1* polymorphisms were determined in a young and healthy population (Example 1).

[0024] The two most common polymorphisms (G596A and A2589G) are both associated with a decreased in vitro ApoA-I mediated efflux of cholesterol from mononuclear phagocytes, which is a typical feature of Tangier disease (Example 2).

[0025] No association of any of the polymorphisms were found with HDL-cholesterol, ApoA-I or any other lipoprotein fraction probably because the effect of the *ABC1* polymorphisms on the protein function is compensated by other factors controlling plasma HDL levels (Example 2 and 4)

[0026] The precise mechanism by which ABC1 promotes cellular lipid efflux is not completely defined. However, recently we could show that ABC1 is expressed on the plasma membrane and the Golgi complex, mediates ApoA-I associated export of cholesterol and phospholipids from the cell, and is regulated by cholesterol flux. Also, our results indicate that ABC1 is involved in lipid export processes involving vesicular budding between the Golgi and the plasma membrane. In addition to a possible function as direct lipid transporter, ABC1 may also be involved in the initiation of signal transduction processes stimulating intracellular lipid transport pathways (40). The finding that pretreatment of monocytes with forskolin, an cAMP elevating agent known to stimulate ApoA-I dependent lipid efflux (28), was able to normalize cholesterol and phospholipid efflux in 596A/A and 2589G/G subjects, may indicate that these gene variants affect the ABC1 function, which is related both to signaling and lipid transport. Since such a defect could be overcome by regulatory signals, this hypothesis may also provide a possible explanation for the lack of an effect on serum HDL levels.

[0027] In summary, these data emphasizes the role of ABC1 in lipid efflux, the first step in reverse cholesterol transport, and can be taken as evidence that common polymorphisms in *ABC1* directly affect cellular lipid homeostasis, which is a key factor in the atherogenetic process.

Frequencies of the identified *ABC1* gene polymorphisms in men with low HDL cholesterol levels and coronary heart disease

[0028] The frequencies of three of the common single nucleotide polymorphisms in *ABC1* (G596A, A2589G, and G3456C) were found to be significantly increased in the population comprised of men having HDL deficiency and established CHD (participants enrolled in the Veterans Affairs Cooperative HDL Cholesterol Intervention Trial ,VA-HIT) relative to control subjects from the Framingham Offspring Study (FOS). Moreover, associations were observed between *ABC1* polymorphisms and status, indicating that wild type alleles were consistently more prevalent in FOS than in VA-HIT but that mutant alleles were consistently more prevalent in VA-HIT (Example 3).

[0029] In summary, these data demonstrate that three of the common variants in the gene encoding ABC1 occur with significantly increased frequency in a population of men having low HDL-C as their primary lipid abnormality and established CHD. These results indicate that common mutations in the *ABC1* play a role in the development of CHD in the general population. These data also provide evidence for a role of *ABC1* in the premature CHD associated with common HDL deficiency states. All three *ABC1* variants are either conserved between human and mouse or are very similar as in the case of A2589G (isoleucine and valine, respectively), suggesting that each has an important functional role.

[0030] Furthermore, the identification of ABC1 as a transporter for IL-1β identifies this gene as a candidate gene for treatment of inflammatory diseases including rheumatoid arthritis and septic shock (27). The cytokine IL-1β is a broadly acting proinflammatory mediator that has been implicated in the pathogenesis of these diseases.

[0031] Moreover, we could demonstrate, that glyburide as an inhibitor of IL-1β secretion inhibits not only Caspase I mediated processing of pro-IL-1β and release of mature IL-1β but simultaneously inhibits ceramide formation from sphingomyelin mediated by neutral sphingomyelinase and thereby releases human fibroblasts from G_2-phase cell cycle arrest. These data provide a further mechanism indicative for a function of ABC1 in signalling and cellular lipid metabolism.

[0032] Autoimmune disorders that are associated with the antiphospholipid syndrome (e.g. lupus erythematodes) can be related to dysregulation of B-cell and T-cell function, aberrant antigen processing, or aberrations in the asymmetric distribution of membrane phospholipids. ABC-transporters are, besides their transport function, candidate genes for phospholipid translocases, floppases and scramblases that regulate phospholipid asymmetry (outer leaflet: PC+SPM; inner leaflet: PS+PE) of biological membranes [11]. There is considerable evidence for a dysregulation of

members of the ABC-transporter family in patient cells. We conclude that these ABC-cassettes are also candidate genes for a genetic basis of antiphospholipid syndromes such as in Lupus erythematodes.

[0033] In summary, our findings emphasize how insight into the pathophysiology of a major disease process may be gleaned from a very rare genetic disorder.

[0034] Lipid disorders represent diseases characterized by changes in the lipid- and lipoproteinconcentrations in the organism. Cardiovascular diseases represent diseases of the heart and vessel system including coronary heart disease and atherosclerosis. Inflammatory diseases include diseases such as psoriasis and lupus erythematodes.

[0035] This invention is directed to the nucleotide sequences of the identified *ABC1* polymorphism variants, the corresponding protein sequences, antibodies, pharmaceutical compositions, diagnostic tests, vectors, and vector host systems according to the claims.

[0036] Our findings identify *ABC1* as a target for the diagnosis of lipid disorders, cardiovascular diseases , and inflammatory diseases.

[0037] Our findings further identify *ABC1* as a target for therapeutic strategies aimed at the prevention of lipid disorders, cardiovascular diseases, and inflammatory diseases.

[0038] The identified *ABC1* polymorphisms may create immunogenic protein sites that can be used for the generation of antibodies. These antibodies can be used for the detection of mutated ABC1-protein and can be used for the diagnosis of lipid disorders, cardiovascular diseases, and inflammatory diseases.

[0039] Therapeutic strategies also include the gene therapy of lipid disorders, cardiovascular diseases, and inflammatory diseases. For this gene therapy, a polynucleotid consisting of a transcription unit is used. The transcription unit consists of at least 9 consecutive nucleotides that are identical or substantial similiar to the ABC1 cDNA sequence. Alternatively, the transcription unit consists of at least 9 consecutive nucleotides leading to the production of antisense RNA of wild type ABC1 or ABC1 containing one or more of the identified polymorphisms. The transcription unit is combined with heterologous transcription regulating sequences, which regulate the transcription in human cells. The heterologous transcription regulating sequences include a promoter that is constitutively active in human cells. Alternatively, the heterologous transcription regulating sequences can contain a promoter that can be induced or repressed in human cells by the addition of a regulatory substance.

[0040] Another therapeutic approach would be the application of antisense molecules or ribozymes or RNA decoys directed towards the *ABC1* transcripts resulting in a modulation of the ABC1 expression. The methodology has been reviewed in (41).

[0041] This invention also includes a vector containing the above described transcription unit and transcription regulating sequences. Preferred vector is a virus, e.g. retrovirus, adenovirus, adeno-associated virus, herpes simplex virus, lentivirus, vaccinia virus, sindbis virus, semliki forest virus.

[0042] Preferred vectors are also plasmids.

[0043] The invention also includes a pharmaceutical preparation that contains the recombinant vectors, e.g. in a colloidal dispersion system. Preferred colloidal dispersion systems are liposomes or polylysin ligands.

[0044] The invention also includes pharmaceutical compositions that contain the above described recombinant vector constructs in combination with nontoxic, inert, pharmaceutically-suited carrier substances. Application of these pharmaceutical compositions can be locally or systemically (e.g. intravenous, intraarterious, intramuscular, subcutane, intradermal, anal, vaginal, nasal, transdermal, intraperitoneal, oral, or as aerosol).

[0045] The invention further includes recombinant host cells, especially recombinant eucaryotic host cells, that contain the above described recombinant vector constructs.

Examples

Example 1

ATP-binding cassette transporter 1 (*ABC1*) gene polymorphisms

Complete cDNA sequence of human *ABC1*

[0046] We have cloned the complete cDNA sequence of human *ABC1* (10). The nucleotide sequence of the complete coding region of the human ABC1 gene is shown in Fig. 1. The open reading frame of 6603 bp encodes a 2201 amino acid protein with a predicted molecular weight of 220 kDa. The protein sequence of the human ABC1 gene is shown in Fig. 2.

Identification of four polymorphisms in human *ABC1*

[0047] Complete sequence analysis (by dye primer sequencing ?) of human *ABC1* in different (how many ?)Tangier

kindred's revealed several polymorphisms, which were also found in healthy control subjects. These are G596A, T1136C, A2589G, and G3456C. All of them change the amino acid sequence of ABC1 and therefore may affect the function of this transporter.

Frequency of polymorphisms in the general population

[0048] In order to determine the frequency of these four polymorphisms in the general population, we have tested 516 healthy blood donors. Table 1 summarizes the characteristics of the blood donors. The allele frequencies shown in table 2 demonstrate that G596A, A2589G and G3456C variants are quite common in a young, healthy population. The G596A polymorphism had the highest prevalence with an estimated frequency of 29.8% for the minor allele (596A), followed by A2589G with 13.7% (2589G), and G3456C with 2.5% (3456C). There was no significant deviation from the Hardy-Weinberg equilibrium. Only only 3 heterozygotes were found for T1136C in our group.

[0049] When we further analyzed the distribution of the polymorphisms in healthy blood donors, we detected a frequent coincidence of the G596A and A2589G polymorphisms (table 3). In homozygote individuals for the minor G596A allele (596A/A; n=41) the frequency of the alleles A and G of the A2589G polymorphism was 73.2% and 26.8% respectively, while in homozygotes for the major G596A allele (596G/G; n=248) the frequencies were 91.3% and 8.7% ($p < 0.001$). This indicates that the two mutations are in linkage disequilibrium in our population. While there was apparently no linkage disequilibrium between G596A and G3456C, the numbers were too small to exclude this with certainty (data not shown).

Methods

[0050] *Sample collection.* Study subjects were recruited from healthy blood donors at the Institute for Clinical Chemistry (department of transfusion medicine) at the University of Regensburg. Samples from 516 individuals who gave consent to genetic analysis at the time of recruitment were available for the study. At the day of sample collection lipid parameters were determined and DNA-extraction was performed. A local review committee approved the Sample collection for genetic analysis.

[0051] *DNA extraction.* All DNA samples were isolated from whole blood with a QIAamp DNA Blood Maxi Kit (Qiagen) according to the manufacturer's recommendations.

[0052] *Detection of polymorphisms.* Genotype analysis was performed on the LightCycler (Roche Diagnostics) as previously described, with minor modifications (29,30). The amplification primers and hybridization probes are listed in table 4. The latter are designated as detection or anchor probes. They are labeled with LightCycler-Red 640 or 705 and fluorescein, respectively. We performed 40 amplification cycles under the following conditions: denaturation at 95°C for 120 s in the first step and 0 s in all subsequent steps, annealing at 57°C for 10 s, extension at 72°C for 15 s. This was followed by a temperature gradient melting curve analysis as described elsewhere (29,30). All detection probes recognize wild-type sequences, with the exception of the probe DET1136/mut, which is complementary to the sequence of the minor allele, i.e. 1136C. In order to assure specificity and accuracy of the LightCycler analysis 100 randomly selected samples were subjected to automated sequencing (ABI Prism 310 Genetic Analyzer), which proved full concordance of the results.

[0053] *Statistical Analysis.* To compare allele frequencies we employed chi-square test.

Example 2

ABC1 gene polymorphisms are associated with impaired ApoA-I mediated cholesterol efflux but not with decreased serum HDL or ApoA-1

[0054] In Tangier patients, mutations in *ABC1* are associated with severely decreased HDL-cholesterol and a lack or marked reduction of ApoA-I induced cholesterol and phospholipid efflux. Therefore, we hypothesized that HDL-cholesterol and ApoA-I levels as well as cholesterol and phospholipid efflux might differ between homozygous wild-type genotypes and carriers of one of the polymorphisms. Since the T1136C polymorphism was only found in heterozygous form in three individuals we did not include T1136C in our statistical analysis.

Analysis of the association of the identified *ABC1* gene polymorphisms with HDL-cholesterol, ApoA-I, or other lipoprotein fractions

[0055] We first analyzed the distribution of lipid and lipoprotein values in individuals with different genotypes. As indicated in table 5 neither heterozygous nor homozygous carriers of the G596A, A2589G, or G3456C polymorphisms had significantly different HDL-cholesterol or ApoA-I levels compared to the homozygous wild type probands. We also

analyzed further parameters of plasmatic lipoprotein metabolism, even though this was not an a priori hypothesis. Total plasma cholesterol, triglycerides, and LDL-cholesterol were also not significantly different among the various genotypes.

Analysis of the association of the identified *ABC1* gene polymorphisms with cholesterol and phospholipid efflux

[0056] We then analyzed cholesterol and phospholipid efflux from mononuclear phagocytes isolated from individuals homozygous for the wild type nucleotide at all four polymorphic sites and from individuals homozygous for the polymorphic allele at position 596 or 2589. Monocytes from subjects homozygous for at least one polymorphic allele were found to have a highly significant reduction in ApoA-I mediated cholesterol efflux (table 6 A). Whereas in cholesterol loaded monocytes from wild type individuals ApoA-I incubation for 17 hrs increases cholesterol efflux to approximately 130% of baseline values, no stimulation of efflux was observed in subjects homozygous for G596A (596A/A) or A2589G (2589G/G). This finding was independent of gender. In order to investigate, whether the lack of cholesterol efflux could be overcome by regulatory mechanisms, cells were pre-incubated with forskolin, a cAMP-elevating agent, which is known to stimulate ApoA-I mediated lipid efflux (28). As shown in table 6 B forskolin treatment normalized cholesterol efflux in subjects with polymorphic alleles.

[0057] A tendency towards reduced ApoA-I mediated efflux of choline containing phospholipids was also correlated with the presence of both variant alleles but did not reach statistical significance (table 6 C). Similar to the ApoA-I mediated cholesterol efflux, forskolin pretreatment abolished the differences between wild type and 596A/A or 2589G/G, respectively (table 6 D).

Methods

[0058] *Lipids and Lipoproteins.* Venous blood was taken from all blood donors after a 12-h fast. The samples were obtained in polypropylene tubes, centrifuged at 4000 x g for 10 min. Cholesterol, triglycerides, and HDL-cholesterol was measured by standard clinical chemistry protocols as previously described. LDL cholesterol was calculated by the Friedewald equation. ApoA-I was determined on a BNA nephelometer (Dade-Behring) with reagents from Dade-Behring.

[0059] *Cellular Lipid Efflux.* Human peripheral blood cells were isolated from lithium-heparin treated blood samples of adult, healthy volunteers. The peripheral blood mononuclear cell fraction was separated by density gradient centrifugation over Ficoll-Histopaque 1.007 (Sigma). Cells positive for CD2, CD7, CD16, CD19 and CD56 were removed by appropriate magnetic beads (Dynal) leaving > 80% pure monocytes. Isolated monocytes were then cultured at a density of $1.5x10^5$ cells/mL in polystyrene culture dishes (100x15mm Petri dish, Falcon) in a serum-free macrophage medium (Macrophage-SFM, Gibco Life Technologies) supplemented with 50ng/mL human recombinant M-CSF (R&D Systems). After an overnight incubation cells were cholesterol loaded for 24 hrs by addition of enzymatically modified LDL (E-LDL; 40 μg/ml), which was prepared as previously described (31). The incubation medium was further supplemented with ^{14}C-cholesterol (1.5 μCi/ml) and ^{3}H-choline (10μCi/ml) in order to label cellular cholesterol and choline containing phospholipids. In a subset of experiments cells were stimulated with 10 μM forskolin during the labeling period. After 24 hrs monocytes were washed and incubated for 17 hrs with or without 10 μg/ml ApoA-I (Sigma) in macrophage medium containing 0.1 % bovine serum albumin and 50ng/mL M-CSF. After incubation the media were removed and centrifuged at 800 x g to precipitate any detached cells. Cells were lysed in 0.2% SDS. Lipids were extracted by liquid extraction according to the method of Bligh and Dyer (13). ^{3}H and ^{14}C radioactivities of the total lipid extracts were measured by liquid scintillation counting in order to distinguish between ^{3}H labeled phospholipids and ^{14}C-cholesterol. Lipid efflux was calculated as percentage of disintegrations per minute (dpm) in medium divided by the sum of dpm recovered from medium and cells. The ApoA-I specific efflux is expressed a percent of basal unstimulated efflux observed in serum-free macrophage medium without ApoA-I.

[0060] *Statistical Analysis.* For comparison of lipid parameters and efflux data between wild type and polymorphisms the two-sample t test was used.

Example 3

Frequencies of the identified *ABC1* gene polymorphisms in men with low HDL cholesterol levels and coronary heart disease

[0061] To adress the question whether the identified polymorphisms in *ABC-1* can provide insight into common HDL deficiency states and CHD risk, we compared the frequencies of three common *ABC1* variants (G596A, A2589G, and G3456C) between two different populations.

[0062] One population consisted of men participating in the Framingham Offspring Study (FOS), a long-term prospective evaluation of cardiovascular disease risk factors (32), whereas the other population consisted of men enrolled in the Veterans Affairs Cooperative HDL Cholesterol Intervention Trial (VA-HIT). VA-HIT, a multicenter study initiated in 1991, was designed to address the hypothesis that increasing HDL-C levels with gemfibrozil therapy would reduce the incidence of death from CHD and nonfatal myocardial infarction in men with established CHD, who had reduced HDL-C concentrations (≤40mg/dL; 1 mmol/L) and normal LDL-C levels (33). The characteristics of these two groups are provided in table 7. Men participating in VA-HIT were older, and slightly heavier, than men in FOS. With respect to plasma lipids, men in VA-HIT had significantly lower concentrations of plasma total cholesterol (-15%), HDL-C (-27%), and LDL-C (-18%), with the greatest difference between the groups noted in HDL-C levels. These differences resulted in a TC:HDL-C ratio which was significantly increased in VA-HIT relative to FOS.in control subjects from the FOS with those of participants in the VA-HIT.

Screening of the FOS and VA-HIT populations for the presence of the identified *ABC1* polymorphisms

[0063] In order to test the hypothesis that *ABC1* polymorphisms may play a role in common HDL deficiency states and, ultimately, CHD risk, genomic DNA from 1,014 men per FOS and VA-HIT population, respectively, was screened for the presence of three common single nucleotide polymorphisms in the *ABC1* gene (G596A, A2589G, and G3456C), using sequence-specific primers with the LightCycler System. The frequency distribution of each *ABC1* polymorphism in the FOS and VA-HIT populations is provided in table 8. With regard to the G596A polymorphism, the frequency of the mutant A allele was significantly increased (P=0.000) in VA-HIT subjects (0.314) relative to FOS (0.266), translating into fewer wild type, but more heterozygous, individuals in the former group. This was similarly the case for the A2589G mutation in which the mutant G allele frequency was 0.162 in VA-HIT versus 0.122 in FOS (P<0.001). The G3456C polymorphism was rare in both the FOS and VA-HIT populations; however, a significant increase (P<0.032) in the frequency of the mutant C allele was observed in VA-HIT subjects (0.038 vs. 0.026). Thus, the frequency of each of these single nucleotide polymorphisms was significantly increased in a population comprised of men having HDL deficiency and established CHD.

Analysis of the associations between each ABC1 polymorphism and status

[0064] We further evaluated these data for the presence of associations between each *ABC1* polymorphism and status. Associations between the G596A and A2589G, G596A and G3456C, and A2589G and G3456C *ABC1* variants in FOS and VA-HIT subjects are presented in tables 9-11, respectively. In each case, a significantly greater proportion of FOS subjects possessed a pair of wild type alleles relative to VA-HIT subjects. In contrast, a significantly greater proportion of VA-HIT subjects possessed either one or two mutant alleles for each *ABC1* variant. These data indicate that wild type alleles were consistently more prevalent in FOS than in VA-HIT, with mutant alleles consistently more prevalent in VA-HIT relative to FOS, suggesting that the occurrence of these *ABC1* variants may be linked.

Methods

[0065] *Subjects.* Subjects were men participating in either the Framingham Offspring (n=1,014) or VA-HIT (n=1,014) studies. The design and methods for FOS have been described elsewhere in detail (32), as have the rationale, design, and results of the VA-HIT study (33). FOS subjects were participants in cycle 4 of FOS and were free of clinical evidence of CHD. For VA-HIT, men were recruited at 20 Veterans Affairs medical centers throughout the United States. Eligibility for the trial required a documented history of CHD, an age of <74 years, an absence of coexisting conditions, an HDL-C level of ≤40 mg/dL (1.0 mmol/L), an LDL-C of ≤140 mg/dL (3.6 mmol/L), and a triglyceride level of <300 mg/dL (3.4 mmol/L). Since DNA was available from 1,014 men participating in the VA-HIT study, an equal number of men participating in cycle 4 of FOS was randomly selected from a total of 1,139 samples available for analysis. Informed consent was obtained from all subjects. Nearly all of the subjects in both groups were Caucasians. Information on alcohol consumption, smoking, blood pressure, body mass index (BMI), and diabetes were available for all subjects enrolled in these studies. Plasma lipid and lipoprotein data used in our analyses were obtained from FOS subjects who were not taking a lipid-lowering medication, while those from the VA-HIT subjects were obtained at baseline, prior to randomization to a treatment (gemfibrozil, 600 mg bid) or placebo group.

[0066] *DNA Isolation.* Genomic DNA was extracted from whole blood samples using either QIAamp mini kits (Qiagen) or Generation Capture Column® kits (Gentra Systems).

[0067] ***Mutation detection.*** Genotype analysis was performed using LightCycler technology (Roche Diagnostics), as described elsewhere in detail (28,34,35). Briefly, this method enables recognition of alterations in a nucleotide sequence through the detection of changes in fluorescence emission, dependent upon energy transfer between fluorophores on two adjacent hybridization probes. Both probes recognize the sequence of a specific amplicon. The de-

tection probe, labeled on its 5' end with LightCycler-Red 640 or 705, overlaps the site of a polymorphic nucleotide, whereas the anchor probe, carrying fluorescein at the 3' end, binds to the amplicon 1 to 3 nucleotides upstream of the detection probe. After PCR in the presence of target DNA, samples are briefly denatured at 95°C, rapidly cooled to 35-40°C, and, in the final step, heated gradually to 70-80°C. Differentiation between a wild type and polymorphic allele is possible, because the detection probe will detach at a different melting temperature dependent upon the presence of a mismatch between the probe and amplicon sequences.

[0068] *Statistical analyses.* To compare the FOS and VA-HIT populations, we employed chi-square tests for categorical measures and two-sample t test for continuous measures, using the SYSTAT statistical package.

Example 4

Analysis of the associations between each *ABC1* polymorphism and plasma lipid profiles in FOS

[0069] Analyses were also performed to test for potential associations between each *ABC1* polymorphism and plasma lipid profiles in FOS. Because the VA-HIT subjects were selected primarily on the basis of plasma lipid values and CHD, we did not test for associations in this group, which was, by design, more homogeneous than that of FOS. In FOS, no statistically significant relationships were detected between any of the 3 *ABC1* polymorphisms and plasma lipids (data not shown). This remained the case after adjustment for familial relationships, age, BMI, smoking, alcohol intake, and apolipoprotein E genotype. Although this result is somewhat incongruous with evidence for a role of ABC1 in the regulation of plasma HDL-C levels (9,11-15), we believe that *ABC1* polymorphisms may directly influence intracellular cholesterol trafficking and, ultimately, reverse cholesterol transport. Support for this hypothesis is provided by the results presented in *Example 2* demonstrating that the homozygosity for either the G596A or A2589G polymorphism is associated with decreased cellular cholesterol efflux in healthy, young subjects. Moreover, because HDL-C levels are modulated by other genetic and environmental factors (7, 36), direct effects of *ABC1* polymorphisms on plasma HDL-C concentrations may not be readily apparent, especially in the heterozygous state.

Methods

[0070] *Measurement of plasma lipids and lipoproteins.* Blood samples were collected from subjects, after a 12-14 h fast, into tubes containing 0.1% EDTA. Plasma was isolated and frozen for subsequent analysis of plasma lipid and lipoprotein concentrations. Plasma total cholesterol and triglyceride concentrations were determined using enzymatic assays (37). Plasma HDL-C concentrations were measured after dextran sulfate-magnesium precipitation of apoB-containing lipoproteins (38), and LDL-C levels were calculated with the equation of Friedewald, Levy, and Fredrickson (39).

[0071] *Statistical analyses.* To evaluate the relationships between ABC1 genotype and plasma lipids, we used analysis of covariance techniques, employing several different models to adjust for potential confounders. Age, BMI, smoking status, alcohol consumption, and apolipoprotein E genotype were included in these models. Loglinear models were used to examine associations between the 3 polymorphisms and status (FOS or VA-HIT). For all 3 models, statistically significant interactions were detected between ABC1 genotype and status, indicating that differences existed between the FOS and VA-HIT groups with regard to the presence of mutant alleles. Specifically, for each ABC1 polymorphism, those in the FOS group were more likely to be categorized as wild type, whereas VA-HIT subjects were more likely to be classified as heterozygous.

SEQUENCE LISTING
<110> Bayer AG

<120> ATP binding cassette transporter 1 (ABC1) gene polymorphisms and uses thereof for the diagnosis and treatment of lipid disorders, cardiovascular diseases and inflammatory diseases

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Leu Arg Gly Val Pro Glu Lys Glu Val Gly Lys Val Gly Glu Trp Ala
      1955                1960                1965

Ile Arg Lys Leu Gly Leu Val Lys Tyr Gly Glu Lys Tyr Ala Gly Asn
   1970                1975                1980

Tyr Ser Gly Gly Asn Lys Arg Lys Leu Ser Thr Ala Met Ala Leu Ile
1985                1990                1995                2000

Gly Gly Pro Pro Val Val Phe Leu Asp Glu Pro Thr Thr Gly Met Asp
                2005                2010                2015

Pro Lys Ala Arg Arg Phe Leu Trp Asn Cys Ala Leu Ser Val Val Lys
            2020                2025                2030

Glu Gly Arg Ser Val Val Leu Thr Ser His Ser Met Glu Glu Cys Glu
      2035                2040                2045

Ala Leu Cys Thr Arg Met Ala Ile Met Val Asn Gly Arg Phe Arg Cys
   2050                2055                2060

Leu Gly Ser Val Gln His Leu Lys Asn Arg Phe Gly Asp Gly Tyr Thr
2065                2070                2075                2080

Ile Val Val Arg Ile Ala Gly Ser Asn Pro Asp Leu Lys Pro Val Gln
                2085                2090                2095

Asp Phe Phe Gly Leu Ala Phe Pro Gly Ser Val Pro Lys Glu Lys His
            2100                2105                2110

Arg Asn Met Leu Gln Tyr Gln Leu Pro Ser Ser Leu Ser Ser Leu Ala
      2115                2120                2125

Arg Ile Phe Ser Ile Leu Ser Gln Ser Lys Lys Arg Leu His Ile Glu
   2130                2135                2140

Asp Tyr Ser Val Ser Gln Thr Thr Leu Asp Gln Val Phe Val Asn Phe
2145                2150                2155                2160

Ala Lys Asp Gln Ser Asp Asp Asp His Leu Lys Asp Leu Ser Leu His
                2165                2170                2175

Lys Asn Gln Thr Val Val Asp Val Ala Val Leu Thr Ser Phe Leu Gln
            2180                2185                2190

Asp Glu Lys Val Lys Glu Ser Tyr Val
      2195                2200
```

Claims

1. A polynucleotide comprising a member selected from the group consisting of:

   (a) a polynucleotide as set forth in SEQ ID NO:1 with the sequence variants G596A or T1136C or A2589G or G3456C or any combination of these variants;

   (b) a polynucleotide fragment of the polynucleotides of (a) containing the variants G596A or T1136C or A2589G or G3456C or any combination of these variants.

2. The polynucleotide of claim 1 wherein the polynucleotide is DNA.

3. A vector containing one or more of the polynucleotides of claim 1 and 2.

4. A host cell containing the vector of claim 3.

5. A process for producing a polypeptide comprising: expressing from the host cell of claim 4 the polypeptide encoded by said DNA.

6. An assay using one of the materials of claims 1 to 4 for the detection of the ABC1 gene transcripts.

7. An assay using one of the materials of claims 1 to 4 for the detection of the ABC1 variants G596A or T1136C or A2589G or G3456C.

8. A polypeptide selected from the group consisting of:

   a) a polypeptide having the deduced amino acid sequence of SEQ ID NO:1 and

   b) fragments, analogs and derivatives thereof containing the sequence variants of claim 2b.

9. An antibody capable to bind to the polypeptide of claim 8.

10. An assay using one of the materials of claims 8 and 9 for the detection of

    (a) the ABC1 protein or fragments, analogs and derivatives thereof,

    (b) the deduced protein or fragments, analogs and derivatives thereof from the ABC1 sequence variants G596A or T1136C or A2589G or G3456C .

11. A diagnostic kit for the detection of the ABC1 gene variants G596A or T1136C or A2589G or G3456C.

12. Modulator of polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

    (a) a polynucleotide as set forth in SEQ ID NO:1 and

    (b) a polynucleotide fragment of the polynucleotide of (a) containing the sequence variants G596A or T1136C or A2589G or G3456C.

13. A pharmaceutical comprising the modulator of claim 12.

14. The modulation of ABC1 transcripts or proteins or fragments thereof by gene therapy.

15. The modulation of ABC1 transcripts or proteins or fragments thereof by antisense or ribozyme technology or RNA decoys.

Figure 1: cDNA of human *ABC1* gene

```
   1  AAACCCCGTA ATTGCGAGCG AGAGTGAGTG GGGCCGGGAC CCGCAGAGCC
  51  GAGCCGACCC TTCTCTCCCG GGCTGCGGCA GGGCAGGGCG GGGAGCTCCG
 101  CGCACCAACA GAGCCGGTTC TCAGGGCGCT TTGCTCCTTG TTTTTTCCCC
 151  GGTTCTGTTT TCTCCCCTTC TCCGGAAGGC TTGTCAAGGG GTAGGAGAAA
 201  GAGACGCAAA CACAAAAGTG GAAAACAGTT AATGACCAGC CACGGCGTCC
 251  CTGCTGTGAG CTCTGGCCGC TGCCTTCCAG GGCTCCCGAG CCACACGCTG
 301  GGGGTGCTGG CTGAGGGAAC ATGGCTTGTT GGCCTCAGCT GAGGTTGCTG
 351  CTGTGGAAGA ACCTCACTTT CAGAAGAAGA caaacatgtc agctgttact
 401  ggaagtggcc tggcctctat ttatcttcct gatcctgatc tctgttcggc
 451  tgagctaccc accctatgaa caacatgaat gccattttcc aaataaagcc
 501  atgccctctg caggaacact tccttgggtt caggggatta tctgtaatgc
 551  caacaacccc tgtttccgtt acccgactcc tggggaggct cccggagttg
 601  ttggaaactt taacaaatcc attgtggctc gcctgttctc agatgctcgg
 651  aggcttcttt tatacagcca gaaagacacc agcatgaagg acatgcgcaa
 701  agttctgaga acattacagc agatcaagaa atccagctca aacttgaagc
 751  ttcaagattt cctggtggac aatgaaacct tctctgggtt cctgtatcac
 801  aacctctctc tcccaaagtc tactgtggac aagatgctga gggctgatgt
 851  cattctccac aaggtatttt tgcaaggcta ccagttacat ttgacaagtc
 901  tgtgcaatgg atcaaaatca gaagagatga ttcaacttgg tgaccaagaa
 951  gtttctgagc tttgtggcct accaagggag aaactggctg cagcagagcg
1001  agtacttcgt tccaacatgg acatcctgaa gccaatcctg agaacactaa
1051  actctacatc tcccttcccg agcaaggagc tggccgaagc cacaaaaaca
1101  ttgctgcata gtcttgggac tctggcccag gagctgttca gcatgagaag
1151  ctggagtgac atgcgacagg aggtgatgtt tctgaccaat gtgaacagct
1201  ccagctcctc cacccaaatc taccaggctg tgtctcgtat tgtctgcggg
1251  catcccgagg gaggggggct gaagatcaag tctctcaact ggtatgagga
1301  caacaactac aaagccctct ttggaggcaa tggcactgag gaagatgctg
1351  aaaccttcta tgacaactct acaactcctt actgcaatga tttgatgaag
1401  aatttggagt ctagtcctct ttcccgcatt atctggaaag ctctgaagcc
1451  gctgctcgtt gggaagatcc tgtatacacc tgacactcca gccacaaggc
1501  aggtcatggc tgaggtgaac aagaccttcc aggaactggc tgtgttccat
1551  gatctggaag gcatgtggga ggaactcagc cccaagatct ggaccttcat
1601  ggagaacagc caagaaatgg accttgtccg gatgctgttg gacagcaggg
1651  acaatgacca cttttgggaa cagcagttgg atggcttaga ttggacagcc
1701  caagacatcg tggcgttttt ggccaagcac ccagaggatg tccagtccag
1751  taatggttct gtgtacacct ggagagaagc tttcaacgag actaaccagg
1801  caatccggac catatctcgc ttcatggagt gtgtcaacct gaacaagcta
1851  gaacccatag caacagaagt ctggctcatc aacaagtcca tggagctgct
1901  ggatgagagg aagttctggg ctggtattgt gttcactgga attactccag
1951  gcagcattga gctgccccat catgtcaagt acaagatccg aatggacatt
2001  gacaatgtgg agaggacaaa taaaatcaag gatgggtact gggaccctgg
2051  tcctcgagct gacccctttg aggacatgcg gtacgtctgg gggggcttcg
2101  cctacttgca ggatgtggtg gagcaggcaa tcatcagggt gctgacgggc
2151  accgagaaga aaactggtgt ctatatgcaa cagatgccct atccctgtta
2201  cgttgatgac atctttctgc gggtgatgag ccggtcaatg cccctcttca
2251  tgacgctggc ctggatttac tcagtggctg tgatcatcaa gggcatcgtg
2301  tatgagaagg aggcacggct gaaagagacc atgcggatca tgggcctgga
2351  caacagcatc ctctggttta gctggttcat tagtagcctc attcctcttc
2401  ttgtgagcgc tggcctgcta gtggtcatcc tgaagttagg aaacctgctg
2451  ccctacagtg atcccagcgt ggtgtttgtc ttcctgtccg tgtttgctgt
2501  ggtgacaatc ctgcagtgct tcctgattag cacactcttc tccagagcca
2551  acctggcagc agcctgtggg ggcatcatct acttcacgct gtacctgccc
2601  tacgtcctgt gtgtggcatg gcaggactac gtgggcttca cactcaagat
2651  cttcgctagc ctgctgtctc ctgtggcttt tgggtttggc tgtgagtact
2701  ttgccctttt tgaggagcag ggcattggag tgcagtggga caacctgttt
2751  gagagtcctg tggaggaaga tggcttcaat ctcaccactt cggtctccat
2801  gatgctgttt gacaccttcc tctatggggt gatgacctgg tacattgagg
2851  ctgtctttcc aggccagtac ggaattccca ggccctggta ttttccttgc
2901  accaagtcct actggtttgg cgaggaaagt gatgagaaga gccaccctgg
2951  ttccaaccag aagagaatat cagaaatctg catggaggag gaacccaccc
3001  acttgaagct gggcgtgtcc attcagaacc tggtaaaagt ctaccgagat
3051  gggatgaagg tggctgtcga tggcctggca ctgaattttt atgagggcca
3101  gatcacctcc ttcctgggcc acaatggagc ggggaagacg accaccatgt
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3151  caatcctgac cgggttgttc cccccgacct cgggcaccgc ctacatcctg
3201  ggaaaagaca ttcgctctga gatgagcacc atccggcaga acctgggggt
3251  ctgtccccag cataacgtgc tgtttgacat gctgactgtc gaagaacaca
3301  tctggttcta tgcccgcttg aaagggctct ctgagaagca cgtgaaggcg
3351  gagatggagc agatggccct ggatgttggt ttgccatcaa gcaagctgaa
3401  aagcaaaaca agccagctgt caggtggaat gcagagaaag ctatctgtgg
3451  ccttggcctt tgtcgggggа tctaaggttg tcattctgga tgaacccaca
3501  gctggtgtgg acccttactc ccgcagggga atatgggagc tgctgctgaa
3551  ataccgacaa ggccgcacca ttattctctc tacacaccac atggatgaag
3601  cggacgtcct gggggacagg attgccatca tctcccatgg gaagctgtgc
3651  tgtgtgggct cctccctgtt tctgaagaac cagctgggaa caggctacta
3701  cctgaccttg gtcaagaaag atgtggaatc ctccctcagt tcctgcagaa
3751  acagtagtag cactgtgtca tacctgaaaa aggaggacag tgtttctcag
3801  agcagttctg atgctggcct gggcagcgac catgagagtg acacgctgac
3851  catcgatgtc tctgctatct ccaacctcat caggaagcat gtgtctgaag
3901  cccggctggt ggaagacata gggcatgagc tgacctatgt gctgccatat
3951  gaagctgcta aggagggagc ctttgtggaa ctctttcatg agattgatga
4001  ccggctctca gacctgggca tttctagtta tggcatctca gagacgaccc
4051  tggaagaaat attcctcaag gtggccgaag agagtggggt ggatgctgag
4101  acctcagatg gtaccttgcc agcaagacga aacaggcggg ccttcgggga
4151  caagcagagc tgtcttcgcc cgttcactga agatgatgct gctgatccaa
4201  atgattctga catagaccca gaatccagag agacagactt gctcagtggg
4251  atggatggca aagggtccta ccaggtgaaa ggctggaaac ttacacagca
4301  acagtttgtg gccctttttgt ggaagagact gctaattgcc agacggagtc
4351  ggaaaggatt ttttgctcag attgtcttgc cagctgtgtt tgtctgcatt
4401  gcccttgtgt tcagcctgat cgtgccaccc tttggcaagt accccagcct
4451  ggaacttcag ccctggatgt acaacgaaca gtacacattt gtcagcaatg
4501  atgctcctga ggacacggga accctggaac tcttaaacgc cctcaccaaa
4551  gaccctggct tcgggacccg ctgtatggaa ggaaacccaa tcccagacac
4601  gccctgccag gcaggggagg aagagtggac cactgcccca gttccccaga
4651  ccatcatgga cctcttccag aatgggaact ggacaatgca gaacccttca
4701  cctgcatgcc agtgtagcag cgacaaaatc aagaagatgc tgcctgtgtg
4751  tcccccaggg gcaggggggc tgcctcctcc acaaagaaaa caaaacactg
4801  cagatatcct tcaggacctg acaggaagaa acatttcgga ttatctggtg
4851  aagacgtatg tgcagatcat agccaaaagc ttaaagaaca agatctgggt
4901  gaatgagttt aggtatggcg gcttttccct gggtgtcagt aatactcaag
4951  cacttcctcc gagtcaagaa gttaatgatg ccaccaaaca aatgaagaaa
5001  cacctaaagc tggccaagga cagttctgca gatcgatttc tcaacagctt
5051  gggaagattt atgacaggac tggacaccag aaataatgtc aaggtgtggt
5101  tcaataacaa gggctggcat gcaatcagct ctttcctgaa tgtcatcaac
5151  aatgccattc tccgggccaa cctgcaaaag ggagagaacc ctagccatta
5201  tggaattact gctttcaatc atcccctgaa tctcaccaag cagcagctct
5251  cagaggtggc tccgatgacc acatcagtgg atgtccttgt gtccatctgt
5301  gtcatctttg caatgtcctt cgtcccagcc agctttgtcg tattcctgat
5351  ccaggagcgg gtcagcaaag caaaacacct gcagttcatc agtggagtga
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5451  gttgtccctg ccacactggt cattatcatc ttcatctgct tccagcagaa
5501  gtcctatgtg tcctccacca atctgcctgt gctagccctt ctacttttgc
5551  tgtatgggtg gtcaatcaca cctctcatgt acccagcctc ctttgtgttc
5601  aagatcccca gcacagccta tgtggtgctc accagcgtga acctcttcat
5651  tggcattaat ggcagcgtgg ccacctttgt gctggagctg ttcaccgaca
5701  ataagctgaa taatatcaat gatatcctga agtccgtgtt cttgatcttc
5751  ccacattttt gcctgggacg agggctcatc gacatggtga aaaaccaggc
5801  aatggctgat gccctggaaa ggtttgggga gaatcgcttt gtgtcaccat
5851  tatcttggga cttggtggga cgaaacctct tcgccatggc cgtggaaggg
5901  gtggtgttct tcctcattac tgttctgatc cagtacagat tcttcatcag
5951  gcccagacct gtaaatgcaa agctatctcc tctgaatgat gaagatgaag
6001  atgtgaggcg ggaaagacag agaattcttg atggtggagg ccagaatgac
6051  atcttagaaa tcaaggagtt gacgaagata tatagaagga agcggaagcc
6101  tgctgttgac aggatttgcg tgggcattcc tcctggtgag tgctttgggc
6151  tcctgggagt taatggggct ggaaaatcat caactttcaa gatgttaaca
6201  ggagatacca ctgttaccag aggagatgct ttccttaaca gaaatagtat
6251  cttatcaaac atccatgaag tacatcagaa catgggctac tgccctcagt
6301  ttgatgccat cacagagctg ttgactggga gagaacacgt ggagttcttt
6351  gccctttga gaggagtccc agagaaagaa gttggcaagg ttggtgagtg
6401  ggcgattcgg aaactgggcc tcgtgaagta tggagaaaaa tatgctggta
```

```
6451  actatagtgg aggcaacaaa cgcaagctct ctacagccat ggctttgatc
6501  ggcgggcctc ctgtggtgtt tctggatgaa cccaccacag gcatggatcc
6551  caaagcccgg cggttcttgt ggaattgtgc cctaagtgtt gtcaaggagg
6601  ggagatcagt agtgcttaca tctcatagta tggaagaatg tgaagctctt
6651  tgcactagga tggcaatcat ggtcaatgga aggttcaggt gccttggcag
6701  tgtccagcat ctaaaaaata ggtttggaga tggttataca atagttgtac
6751  gaatagcagg gtccaacccg gacctgaagc ctgtccagga tttctttgga
6801  cttgcatttc ctggaagtgt tccaaaagag aaacaccgga acatgctaca
6851  ataccagctt ccatcttcat tatcttctct ggccaggata ttcagcatcc
6901  tctcccagag caaaaagcga ctccacatag aagactactc tgtttctcag
6951  acaacacttg accaagtatt tgtgaacttt gccaaggacc aaagtgatga
7001  tgaccactta aaagacctct cattacacaa aaaccagaca gtagtggacg
7051  ttgcagttct cacatctttt ctacaggatg agaaagtgaa agaaagctat
7101  gtatgaagaa tcctgttcat acggggtggc tgaaagtaaa gagggactag
7151  actttccttt gcaccatgtg aagtgttgtg gagaaaagag ccagaagttg
7201  atgtgggaag aagtaaactg gatactgtac tgatactatt caatgcaatg
7251  caattcaatg
```

Figure 2: human *ABC1* gene – protein translation

```
     AAACCCCGTAATTGCGAGCGAGAGTGAGTGGGGCCGGGACCCGCAGAGCC
   1 ---------+---------+---------+---------+---------+  50

     GAGCCGACCCTTCTCTCCCGGGCTGCGGCAGGGCAGGGCGGGGAGCTCCG
  51 ---------+---------+---------+---------+---------+ 100

     CGCACCAACAGAGCCGGTTCTCAGGGCGCTTTGCTCCTTGTTTTTTCCCC
 101 ---------+---------+---------+---------+---------+ 150

     GGTTCTGTTTTCTCCCCTTCTCCGGAAGGCTTGTCAAGGGGTAGGAGAAA
 151 ---------+---------+---------+---------+---------+ 200

     GAGACGCAAACACAAAAGTGGAAAACAGTTAATGACCAGCCACGGCGTCC
 201 ---------+---------+---------+---------+---------+ 250

     CTGCTGTGAGCTCTGGCCGCTGCCTTCCAGGGCTCCCGAGCCACACGCTG
 251 ---------+---------+---------+---------+---------+ 300

     GGGGTGCTGGCTGAGGGAACATGGCTTGTTGGCCTCAGCTGAGGTTGCTG
 301 ---------+---------+---------+---------+---------+ 350
                          M   A   C   W   P   Q   L   R   L   L   -

     CTGTGGAAGAACCTCACTTTCAGAAGAAGAcaaacatgtcagctgttact
 351 ---------+---------+---------+---------+---------+ 400
      L   W   K   N   L   T   F   R   R   R   Q   T   C   Q   L   L   L   -

     ggaagtggcctggcctctatttatcttcctgatcctgatctctgttcggc
 401 ---------+---------+---------+---------+---------+ 450
       E   V   A   W   P   L   F   I   F   L   I   L   I   S   V   R   L   -

     tgagctacccaccctatgaacaacatgaatgccattttccaaataaagcc
 451 ---------+---------+---------+---------+---------+ 500
         S   Y   P   P   Y   E   Q   H   E   C   H   F   P   N   K   A   -

     atgccctctgcaggaacacttccttgggttcaggggattatctgtaatgc
 501 ---------+---------+---------+---------+---------+ 550
      M   P   S   A   G   T   L   P   W   V   Q   G   I   I   C   N   A   -

     caacaacccctgtttccgttacccgactcctggggaggctcccggagttg
 551 ---------+---------+---------+---------+---------+ 600
       N   N   P   C   F   R   Y   P   T   P   G   E   A   P   G   V   V   -
```

```
    ttggaaactttaacaaatccattgtggctcgcctgttctcagatgctcgg
601 ---------+---------+---------+---------+---------+ 650
      G   N   F   N   K   S   I   V   A   R   L   F   S   D   A   R   -

    aggcttcttttatacagccagaaagacaccagcatgaaggacatgcgcaa
651 ---------+---------+---------+---------+---------+ 700
    R   L   L   L   Y   S   Q   K   D   T   S   M   K   D   M   R   K   -

    agttctgagaacattacagcagatcaagaaatccagctcaaacttgaagc
701 ---------+---------+---------+---------+---------+ 750
     V   L   R   T   L   Q   Q   I   K   K   S   S   S   N   L   K   L   -

    ttcaagatttcctggtggacaatgaaaccttctctgggttcctgtatcac
751 ---------+---------+---------+---------+---------+ 800
      Q   D   F   L   V   D   N   E   T   F   S   G   F   L   Y   H   -

    aacctctctctcccaaagtctactgtggacaagatgctgagggctgatgt
801 ---------+---------+---------+---------+---------+ 850
      N   L   S   L   P   K   S   T   V   D   K   M   L   R   A   D   V   -

    cattctccacaaggtatttttgcaaggctaccagttacatttgacaagtc
851 ---------+---------+---------+---------+---------+ 900
     I   L   H   K   V   F   L   Q   G   Y   Q   L   H   L   T   S   L   -

    tgtgcaatggatcaaaatcagaagagatgattcaacttggtgaccaagaa
901 ---------+---------+---------+---------+---------+ 950
      C   N   G   S   K   S   E   E   M   I   Q   L   G   D   Q   E   -

    gtttctgagctttgtggcctaccaagggagaaactggctgcagcagagcg
951 ---------+---------+---------+---------+---------+ 1000
    V   S   E   L   C   G   L   P   R   E   K   L   A   A   A   E   R   -

    agtacttcgttccaacatggacatcctgaagccaatcctgagaacactaa
1001 ---------+---------+---------+---------+---------+ 1050
     V   L   R   S   N   M   D   I   L   K   P   I   L   R   T   L   N   -

    actctacatctcccttcccgagcaaggagctggccgaagccacaaaaaca
1051 ---------+---------+---------+---------+---------+ 1100
      S   T   S   P   F   P   S   K   E   L   A   E   A   T   K   T   -

    ttgctgcatagtcttgggactctggcccaggagctgttcagcatgagaag
1101 ---------+---------+---------+---------+---------+ 1150
    L   L   H   S   L   G   T   L   A   Q   E   L   F   S   M   R   S   -

    ctggagtgacatgcgacaggaggtgatgtttctgaccaatgtgaacagct
1151 ---------+---------+---------+---------+---------+ 1200
     W   S   D   M   R   Q   E   V   M   F   L   T   N   V   N   S   S   -

    ccagctcctccacccaaatctaccaggctgtgtctcgtattgtctgcggg
1201 ---------+---------+---------+---------+---------+ 1250
      S   S   S   T   Q   I   Y   Q   A   V   S   R   I   V   C   G   -

    catcccgagggaggggggctgaagatcaagtctctcaactggtatgagga
1251 ---------+---------+---------+---------+---------+ 1300
    H   P   E   G   G   G   L   K   I   K   S   L   N   W   Y   E   D   -

    caacaactacaaagccctctttggaggcaatggcactgaggaagatgctg
1301 ---------+---------+---------+---------+---------+ 1350
     N   N   Y   K   A   L   F   G   G   N   G   T   E   E   D   A   E   -

    aaaccttctatgacaactctacaactccttactgcaatgatttgatgaag
1351 ---------+---------+---------+---------+---------+ 1400
      T   F   Y   D   N   S   T   T   P   Y   C   N   D   L   M   K   -
```

```
     aatttggagtctagtcctctttcccgcattatctggaaagctctgaagcc
1401 ---------+---------+---------+---------+---------+---------+ 1450
     N  L  E  S  S  P  L  S  R  I  I  W  K  A  L  K  P  -

     gctgctcgttgggaagatcctgtatacacctgacactccagccacaaggc
1451 ---------+---------+---------+---------+---------+---------+ 1500
      L  L  V  G  K  I  L  Y  T  P  D  T  P  A  T  R  Q  -

     aggtcatggctgaggtgaacaagaccttccaggaactggctgtgttccat
1501 ---------+---------+---------+---------+---------+---------+ 1550
        V  M  A  E  V  N  K  T  F  Q  E  L  A  V  F  H  -
     gatctggaaggcatgtgggaggaactcagccccaagatctggaccttcat
1551 ---------+---------+---------+---------+---------+---------+ 1600
     D  L  E  G  M  W  E  E  L  S  P  K  I  W  T  F  M  -

     ggagaacagccaagaaatggaccttgtccggatgctgttggacagcaggg
1601 ---------+---------+---------+---------+---------+---------+ 1650
      E  N  S  Q  E  M  D  L  V  R  M  L  L  D  S  R  D  -

     acaatgaccacttttgggaacagcagttggatggcttagattggacagcc
1651 ---------+---------+---------+---------+---------+---------+ 1700
       N  D  H  F  W  E  Q  Q  L  D  G  L  D  W  T  A  -

     caagacatcgtggcgttttggccaagcacccagaggatgtccagtccag
1701 ---------+---------+---------+---------+---------+---------+ 1750
     Q  D  I  V  A  F  L  A  K  H  P  E  D  V  Q  S  S  -

     taatggttctgtgtacacctggagagaagctttcaacgagactaaccagg
1751 ---------+---------+---------+---------+---------+---------+ 1800
      N  G  S  V  Y  T  W  R  E  A  F  N  E  T  N  Q  A  -

     caatccggaccatatctcgcttcatggagtgtgtcaacctgaacaagcta
1801 ---------+---------+---------+---------+---------+---------+ 1850
       I  R  T  I  S  R  F  M  E  C  V  N  L  N  K  L  -

     gaacccatagcaacagaagtctggctcatcaacaagtccatggagctgct
1851 ---------+---------+---------+---------+---------+---------+ 1900
     E  P  I  A  T  E  V  W  L  I  N  K  S  M  E  L  L  -

     ggatgagaggaagttctgggctggtattgtgttcactggaattactccag
1901 ---------+---------+---------+---------+---------+---------+ 1950
      D  E  R  K  F  W  A  G  I  V  F  T  G  I  T  P  G  -

     gcagcattgagctgccccatcatgtcaagtacaagatccgaatggacatt
1951 ---------+---------+---------+---------+---------+---------+ 2000
       S  I  E  L  P  H  H  V  K  Y  K  I  R  M  D  I  -

     gacaatgtggagaggacaaataaaatcaaggatgggtactgggaccctgg
2001 ---------+---------+---------+---------+---------+---------+ 2050
     D  N  V  E  R  T  N  K  I  K  D  G  Y  W  D  P  G  -

     tcctcgagctgacccctttgaggacatgcggtacgtctgggggggcttcg
2051 ---------+---------+---------+---------+---------+---------+ 2100
      P  R  A  D  P  F  E  D  M  R  Y  V  W  G  G  F  A  -

     cctacttgcaggatgtggtggagcaggcaatcatcagggtgctgacgggc
2101 ---------+---------+---------+---------+---------+---------+ 2150
       Y  L  Q  D  V  V  E  Q  A  I  I  R  V  L  T  G  -

     accgagaagaaaactggtgtctatatgcaacagatgccctatccctgtta
2151 ---------+---------+---------+---------+---------+---------+ 2200
     T  E  K  K  T  G  V  Y  M  Q  Q  M  P  Y  P  C  Y  -

     cgttgatgacatctttctgcgggtgatgagccggtcaatgcccctcttca
2201 ---------+---------+---------+---------+---------+---------+ 2250
```

```
      V  D  D  I  F  L  R  V  M  S  R  S  M  P  L  F  M -
     tgacgctggcctggatttactcagtggctgtgatcatcaagggcatcgtg
2251 ---------+---------+---------+---------+---------+ 2300
       T  L  A  W  I  Y  S  V  A  V  I  I  K  G  I  V  -

     tatgagaaggaggcacggctgaaagagaccatgcggatcatgggcctgga
2301 ---------+---------+---------+---------+---------+ 2350
     Y  E  K  E  A  R  L  K  E  T  M  R  I  M  G  L  D -

     caacagcatcctctggtttagctggttcattagtagcctcattcctcttc
2351 ---------+---------+---------+---------+---------+ 2400
      N  S  I  L  W  F  S  W  F  I  S  S  L  I  P  L  L -

     ttgtgagcgctggcctgctagtggtcatcctgaagttaggaaacctgctg
2401 ---------+---------+---------+---------+---------+ 2450
       V  S  A  G  L  L  V  V  I  L  K  L  G  N  L  L  -

     ccctacagtgatcccagcgtggtgtttgtcttcctgtccgtgtttgctgt
2451 ---------+---------+---------+---------+---------+ 2500
     P  Y  S  D  P  S  V  V  F  V  F  L  S  V  F  A  V -

     ggtgacaatcctgcagtgcttcctgattagcacactcttcccagagcca
2501 ---------+---------+---------+---------+---------+ 2550
      V  T  I  L  Q  C  F  L  I  S  T  L  F  S  R  A  N -

     acctggcagcagcctgtgggggcatcatctacttcacgctgtacctgccc
2551 ---------+---------+---------+---------+---------+ 2600
       L  A  A  A  C  G  G  I  I  Y  F  T  L  Y  L  P  -

     tacgtcctgtgtgtggcatggcaggactacgtgggcttcacactcaagat
2601 ---------+---------+---------+---------+---------+ 2650
     Y  V  L  C  V  A  W  Q  D  Y  V  G  F  T  L  K  I -

     cttcgctagcctgctgtctcctgtggcttttgggtttggctgtgagtact
2651 ---------+---------+---------+---------+---------+ 2700
      F  A  S  L  L  S  P  V  A  F  G  F  G  C  E  Y  F -

     ttgccctttttgaggagcagggcattggagtgcagtgggacaacctgttt
2701 ---------+---------+---------+---------+---------+ 2750
       A  L  F  E  E  Q  G  I  G  V  Q  W  D  N  L  F  -

     gagagtcctgtggaggaagatggcttcaatctcaccacttcggtctccat
2751 ---------+---------+---------+---------+---------+ 2800
     E  S  P  V  E  E  D  G  F  N  L  T  T  S  V  S  M -

     gatgctgtttgacaccttcctctatggggtgatgacctggtacattgagg
2801 ---------+---------+---------+---------+---------+ 2850
      M  L  F  D  T  F  L  Y  G  V  M  T  W  Y  I  E  A -

     ctgtctttccaggccagtacggaattcccaggccctggtattttccttgc
2851 ---------+---------+---------+---------+---------+ 2900
       V  F  P  G  Q  Y  G  I  P  R  P  W  Y  F  P  C  -

     accaagtcctactggtttggcgaggaaagtgatgagaagagccaccctgg
2901 ---------+---------+---------+---------+---------+ 2950
     T  K  S  Y  W  F  G  E  E  S  D  E  K  S  H  P  G -
     ttccaaccagaagagaatatcagaaatctgcatggaggaggaacccaccc
2951 ---------+---------+---------+---------+---------+ 3000
      S  N  Q  K  R  I  S  E  I  C  M  E  E  E  P  T  H -

     acttgaagctgggcgtgtccattcagaacctggtaaaagtctaccgagat
3001 ---------+---------+---------+---------+---------+ 3050
       L  K  L  G  V  S  I  Q  N  L  V  K  V  Y  R  D  -

     gggatgaaggtggctgtcgatggcctggcactgaattttatgagggcca
3051 ---------+---------+---------+---------+---------+ 3100
```

```
     G  M  K  V  A  V  D  G  L  A  L  N  F  Y  E  G  Q  -

     gatcacctccttcctgggccacaatggagcggggaagacgaccaccatgt
3101 ---------+---------+---------+---------+---------+---------+ 3150
      I  T  S  F  L  G  H  N  G  A  G  K  T  I  T  M  S  -

     caatcctgaccgggttgttccccccgacctcgggcaccgcctacatcctg
3151 ---------+---------+---------+---------+---------+---------+ 3200
        I  L  T  G  L  F  P  P  T  S  G  T  A  Y  I  L  -

     ggaaaagacattcgctctgagatgagcaccatccggcagaacctgggggt
3201 ---------+---------+---------+---------+---------+---------+ 3250
     G  K  D  I  R  S  E  M  S  T  I  R  Q  N  L  G  V  -

     ctgtccccagcataacgtgctgtttgacatgctgactgtcgaagaacaca
3251 ---------+---------+---------+---------+---------+---------+ 3300
      C  P  Q  H  N  V  L  F  D  M  L  T  V  E  E  H  I  -

     tctggttctatgcccgcttgaaagggctctctgagaagcacgtgaaggcg
3301 ---------+---------+---------+---------+---------+---------+ 3350
        W  F  Y  A  R  L  K  G  L  S  E  K  H  V  K  A  -

     gagatggagcagatggccctggatgttggtttgccatcaagcaagctgaa
3351 ---------+---------+---------+---------+---------+---------+ 3400
     E  M  E  Q  M  A  L  D  V  G  L  P  S  S  K  L  K  -

     aagcaaaacaagccagctgtcaggtggaatgcagagaaagctatctgtgg
3401 ---------+---------+---------+---------+---------+---------+ 3450
      S  K  T  S  Q  L  S  G  G  M  Q  R  K  L  S  V  A  -

     ccttggcctttgtcgggggatctaaggttgtcattctggatgaacccaca
3451 ---------+---------+---------+---------+---------+---------+ 3500
        L  A  F  V  G  G  S  K  V  V  I  L  D  E  P  T  -

     gctggtgtggacccttactcccgcaggggaatatgggagctgctgctgaa
3501 ---------+---------+---------+---------+---------+---------+ 3550
     A  G  V  D  P  Y  S  R  R  G  I  W  E  L  L  L  K  -

     ataccgacaaggccgcaccattattctctctacacaccacatggatgaag
3551 ---------+---------+---------+---------+---------+---------+ 3600
      Y  R  Q  G  R  T  I  I  L  S  T  H  H  M  D  E  A  -

     cggacgtcctgggggacaggattgccatcatctcccatgggaagctgtgc
3601 ---------+---------+---------+---------+---------+---------+ 3650
        D  V  L  G  D  R  I  A  I  I  S  H  G  K  L  C  -
     tgtgtgggctcctccctgtttctgaagaaccagctgggaacaggctacta
3651 ---------+---------+---------+---------+---------+---------+ 3700
     C  V  G  S  S  L  F  L  K  N  Q  L  G  T  G  Y  Y  -

     cctgaccttggtcaagaaagatgtggaatcctccctcagttcctgcagaa
3701 ---------+---------+---------+---------+---------+---------+ 3750
c       L  T  L  V  K  K  D  V  E  S  S  L  S  S  C  R  N  -

     acagtagtagcactgtgtcatacctgaaaaaggaggacagtgtttctcag
3751 ---------+---------+---------+---------+---------+---------+ 3800
        S  S  S  T  V  S  Y  L  K  K  E  D  S  V  S  Q  -

     agcagttctgatgctggcctgggcagcgaccatgagagtgacacgctgac
3801 ---------+---------+---------+---------+---------+---------+ 3850
     S  S  S  D  A  G  L  G  S  D  H  E  S  D  T  L  T  -

     catcgatgtctctgctatctccaacctcatcaggaagcatgtgtctgaag
3851 ---------+---------+---------+---------+---------+---------+ 3900
      I  D  V  S  A  I  S  N  L  I  R  K  H  V  S  E  A  -

     cccggctggtggaagacatagggcatgagctgacctatgtgctgccatat
```

```
3901 ---------+---------+---------+---------+---------+---------+ 3950
       R   L   V   E   D   I   G   H   E   L   T   Y   V   L   P   Y   -

     gaagctgctaaggagggagcctttgtggaactctttcatgagattgatga
3951 ---------+---------+---------+---------+---------+---------+ 4000
     E   A   A   K   E   G   A   F   V   E   L   F   H   E   I   D   D   -

     ccggctctcagacctgggcatttctagttatggcatctcagagacgaccc
4001 ---------+---------+---------+---------+---------+---------+ 4050
      R   L   S   D   L   G   I   S   S   Y   G   I   S   E   T   T   L -

     tggaagaaatattcctcaaggtggccgaagagagtggggtggatgctgag
4051 ---------+---------+---------+---------+---------+---------+ 4100
       E   E   I   F   L   K   V   A   E   E   S   G   V   D   A   E   -

     acctcagatggtaccttgccagcaagacgaaacaggcgggccttcgggga
4101 ---------+---------+---------+---------+---------+---------+ 4150
     T   S   D   G   T   L   P   A   R   R   N   R   R   A   F   G   D   -

     caagcagagctgtcttcgcccgttcactgaagatgatgctgctgatccaa
4151 ---------+---------+---------+---------+---------+---------+ 4200
      K   Q   S   C   L   R   P   F   T   E   D   D   A   A   D   P   N -

     atgattctgacatagacccagaatccagagagacagacttgctcagtggg
4201 ---------+---------+---------+---------+---------+---------+ 4250
       D   S   D   I   D   P   E   S   R   E   T   D   L   L   S   G   -

     atggatggcaaagggtcctaccaggtgaaaggctggaaacttacacagca
4251 ---------+---------+---------+---------+---------+---------+ 4300
     M   D   G   K   G   S   Y   Q   V   K   G   W   K   L   T   Q   Q   -

     acagtttgtggccctttgtggaagagactgctaattgccagacggagtc
4301 ---------+---------+---------+---------+---------+---------+ 4350
      Q   F   V   A   L   L   W   K   R   L   L   I   A   R   R   S   R
     ggaaaggatttttgctcagattgtcttgccagctgtgtttgtctgcatt
4351 ---------+---------+---------+---------+---------+---------+ 4400
       K   G   F   F   A   Q   I   V   L   P   A   V   F   V   C   I   -

     gcccttgtgttcagcctgatcgtgccacccttggcaagtaccccagcct
4401 ---------+---------+---------+---------+---------+---------+ 4450
     A   L   V   F   S   L   I   V   P   P   F   G   K   Y   P   S   L   -

     ggaacttcagccctggatgtacaacgaacagtacacatttgtcagcaatg
4451 ---------+---------+---------+---------+---------+---------+ 4500
      E   L   Q   P   W   M   Y   N   E   Q   Y   T   F   V   S   N   D -

     atgctcctgaggacacgggaaccctggaactcttaaacgccctcaccaaa
4501 ---------+---------+---------+---------+---------+---------+ 4550
       A   P   E   D   T   G   T   L   E   L   L   N   A   L   T   K   -

     gaccctggcttcgggacccgctgtatggaaggaaacccaatcccagacac
4551 ---------+---------+---------+---------+---------+---------+ 4600
     D   P   G   F   G   T   R   C   M   E   G   N   P   I   P   D   T   -

     gccctgccaggcaggggaggaagagtggaccactgccccagttccccaga
4601 ---------+---------+---------+---------+---------+---------+ 4650
      P   C   Q   A   G   E   E   E   W   T   T   A   P   V   P   Q   T -

     ccatcatggacctcttccagaatgggaactggacaatgcagaacccttca
4651 ---------+---------+---------+---------+---------+---------+ 4700
       I   M   D   L   F   Q   N   G   N   W   T   M   Q   N   P   S   -

     cctgcatgccagtgtagcagcgacaaaatcaagaagatgctgcctgtgtg
4701 ---------+---------+---------+---------+---------+---------+ 4750
     P   A   C   Q   C   S   S   D   K   I   K   K   M   L   P   V   C   -
```

```
     tcccccaggggcaggggggctgcctcctccacaaagaaaacaaaacactg
4751 ---------+---------+---------+---------+---------+ 4800
      P  P  G  A  G  G  L  P  P  P  Q  R  K  Q  N  T  A -

     cagatatccttcaggacctgacaggaagaaacatttcggattatctggtg
4801 ---------+---------+---------+---------+---------+ 4850
        D  I  L  Q  D  L  T  G  R  N  I  S  D  Y  L  V   -

     aagacgtatgtgcagatcatagccaaaagcttaaagaacaagatctgggt
4851 ---------+---------+---------+---------+---------+ 4900
     K  T  Y  V  Q  I  I  A  K  S  L  K  N  K  I  W  V  -

     gaatgagtttaggtatggcggcttttccctgggtgtcagtaatactcaag
4901 ---------+---------+---------+---------+---------+ 4950
      N  E  F  R  Y  G  G  F  S  L  G  V  S  N  T  Q  A -

     cacttcctccgagtcaagaagttaatgatgccaccaaacaaatgaagaaa
4951 ---------+---------+---------+---------+---------+ 5000
        L  P  P  S  Q  E  V  N  D  A  T  K  Q  M  K  K   -

     cacctaaagctggccaaggacagttctgcagatcgatttctcaacagctt
5001 ---------+---------+---------+---------+---------+ 5050
     H  L  K  L  A  K  D  S  S  A  D  R  F  L  N  S  L  -
     gggaagatttatgacaggactggacaccagaaataatgtcaaggtgtggt
5051 ---------+---------+---------+---------+---------+ 5100
      G  R  F  M  T  G  L  D  T  R  N  N  V  K  V  W  F -

     tcaataacaagggctggcatgcaatcagctctttcctgaatgtcatcaac
5101 ---------+---------+---------+---------+---------+ 5150
        N  N  K  G  W  H  A  I  S  S  F  L  N  V  I  N   -

     aatgccattctccgggccaacctgcaaaagggagagaaccctagccatta
5151 ---------+---------+---------+---------+---------+ 5200
     N  A  I  L  R  A  N  L  Q  K  G  E  N  P  S  H  Y  -

     tggaattactgctttcaatcatcccctgaatctcaccaagcagcagctct
5201 ---------+---------+---------+---------+---------+ 5250
      G  I  T  A  F  N  H  P  L  N  L  T  K  Q  Q  L  S -

     cagaggtggctccgatgaccacatcagtggatgtccttgtgtccatctgt
5251 ---------+---------+---------+---------+---------+ 5300
        E  V  A  P  M  T  T  S  V  D  V  L  V  S  I  C   -

     gtcatctttgcaatgtccttcgtcccagccagctttgtcgtattcctgat
5301 ---------+---------+---------+---------+---------+ 5350
     V  I  F  A  M  S  F  V  P  A  S  F  V  V  F  L  I  -

     ccaggagcgggtcagcaaagcaaaacacctgcagttcatcagtggagtga
5351 ---------+---------+---------+---------+---------+ 5400
        Q  E  R  V  S  K  A  K  H  L  Q  F  I  S  G  V  K -

     agcctgtcatctactggctctctaattttgtctgggatatgtgcaattac
5401 ---------+---------+---------+---------+---------+ 5450
        P  V  I  Y  W  L  S  N  F  V  W  D  M  C  N  Y   -

     gttgtccctgccacactggtcattatcatcttcatctgcttccagcagaa
5451 ---------+---------+---------+---------+---------+ 5500
     V  V  P  A  T  L  V  I  I  I  F  I  C  F  Q  Q  K  -

     gtcctatgtgtcctccaccaatctgcctgtgctagcccttctacttttgc
5501 ---------+---------+---------+---------+---------+ 5550
      S  Y  V  S  S  T  N  L  P  V  L  A  L  L  L  L  L -

     tgtatgggtggtcaatcacacctctcatgtacccagcctcctttgtgttc
5551 ---------+---------+---------+---------+---------+ 5600
        Y  G  W  S  I  T  P  L  M  Y  P  A  S  F  V  F   -
```

```
     aagatccccagcacagcctatgtggtgctcaccagcgtgaacctcttcat
5601 ---------+---------+---------+---------+---------+---------+ 5650
     K  I  P  S  T  A  Y  V  V  L  T  S  V  N  L  F  I  -

     tggcattaatggcagcgtggccacctttgtgctggagctgttcaccgaca
5651 ---------+---------+---------+---------+---------+---------+ 5700
      G  I  N  G  S  V  A  T  F  V  L  E  L  F  T  D  N -

     ataagctgaataatatcaatgatatcctgaagtccgtgttcttgatcttc
5701 ---------+---------+---------+---------+---------+---------+ 5750
        K  L  N  N  I  N  D  I  L  K  S  V  F  L  I  F  -
     ccacattttgcctgggacgagggctcatcgacatggtgaaaaaccaggc
5751 ---------+---------+---------+---------+---------+---------+ 5800
     P  H  F  C  L  G  R  G  L  I  D  M  V  K  N  Q  A  -

     aatggctgatgccctggaaaggtttggggagaatcgctttgtgtcaccat
5801 ---------+---------+---------+---------+---------+---------+ 5850
      M  A  D  A  L  E  R  F  G  E  N  R  F  V  S  P  L -

     tatcttgggacttggtgggacgaaacctcttcgccatggccgtggaaggg
5851 ---------+---------+---------+---------+---------+---------+ 5900
        S  W  D  L  V  G  R  N  L  F  A  M  A  V  E  G  -

     gtggtgttcttcctcattactgttctgatccagtacagattcttcatcag
5901 ---------+---------+---------+---------+---------+---------+ 5950
     V  V  F  F  L  I  T  V  L  I  Q  Y  R  F  F  I  R  -

     gcccagacctgtaaatgcaaagctatctcctctgaatgatgaagatgaag
5951 ---------+---------+---------+---------+---------+---------+ 6000
      P  R  P  V  N  A  K  L  S  P  L  N  D  E  D  E  D -

     atgtgaggcgggaaagacagagaattcttgatggtggaggccagaatgac
6001 ---------+---------+---------+---------+---------+---------+ 6050
        V  R  R  E  R  Q  R  I  L  D  G  G  G  Q  N  D  -

     atcttagaaatcaaggagttgacgaagatatatagaaggaagcggaagcc
6051 ---------+---------+---------+---------+---------+---------+ 6100
     I  L  E  I  K  E  L  T  K  I  Y  R  R  K  R  K  P  -

     tgctgttgacaggatttgcgtgggcattcctcctggtgagtgctttgggc
6101 ---------+---------+---------+---------+---------+---------+ 6150
      A  V  D  R  I  C  V  G  I  P  P  G  E  C  F  G  L -

     tcctgggagttaatggggctggaaaatcatcaactttcaagatgttaaca
6151 ---------+---------+---------+---------+---------+---------+ 6200
        L  G  V  N  G  A  G  K  S  S  T  F  K  M  L  T  -

     ggagataccactgttaccagaggagatgctttccttaacagaaatagtat
6201 ---------+---------+---------+---------+---------+---------+ 6250
     G  D  T  T  V  T  R  G  D  A  F  L  N  R  N  S  I  -

     cttatcaaacatccatgaagtacatcagaacatgggctactgccctcagt
6251 ---------+---------+---------+---------+---------+---------+ 6300
      L  S  N  I  H  E  V  H  Q  N  M  G  Y  C  P  Q  F -

     ttgatgccatcacagagctgttgactgggagagaacacgtggagttcttt
6301 ---------+---------+---------+---------+---------+---------+ 6350
        D  A  I  T  E  L  L  T  G  R  E  H  V  E  F  F  -

     gcccttttgagaggagtcccagagaaagaagttggcaaggttggtgagtg
6351 ---------+---------+---------+---------+---------+---------+ 6400
     A  L  L  R  G  V  P  E  K  E  V  G  K  V  G  E  W  -

     ggcgattcggaaactgggcctcgtgaagtatggagaaaaatatgctggta
6401 ---------+---------+---------+---------+---------+---------+ 6450
```

```
      A  I  R  K  L  G  L  V  K  Y  G  E  K  Y  A  G  N -
     actatagtggaggcaacaaacgcaagctctctacagccatggctttgatc
6451 ---------+---------+---------+---------+---------+---------+ 6500
       Y  S  G  G  N  K  R  K  L  S  T  A  M  A  L  I  -

     ggcgggcctcctgtggtgtttctggatgaacccaccacaggcatggatcc
6501 ---------+---------+---------+---------+---------+---------+ 6550
     G  G  P  P  V  V  F  L  D  E  P  T  T  G  M  D  P  -

     caaagcccggcggttcttgtggaattgtgccctaagtgttgtcaaggagg
6551 ---------+---------+---------+---------+---------+---------+ 6600
      K  A  R  R  F  L  W  N  C  A  L  S  V  V  K  E  G -

     ggagatcagtagtgcttacatctcatagtatggaagaatgtgaagctctt
6601 ---------+---------+---------+---------+---------+---------+ 6650
       R  S  V  V  L  T  S  H  S  M  E  E  C  E  A  L  -

     tgcactaggatggcaatcatggtcaatggaaggttcaggtgccttggcag
6651 ---------+---------+---------+---------+---------+---------+ 6700
     C  T  R  M  A  I  M  V  N  G  R  F  R  C  L  G  S  -

     tgtccagcatctaaaaaataggtttggagatggttatacaatagttgtac
6701 ---------+---------+---------+---------+---------+---------+ 6750
      V  Q  H  L  K  N  R  F  G  D  G  Y  T  I  V  V  R -

     gaatagcagggtccaacccggacctgaagcctgtccaggatttctttgga
6751 ---------+---------+---------+---------+---------+---------+ 6800
       I  A  G  S  N  P  D  L  K  P  V  Q  D  F  F  G  -

     cttgcatttcctggaagtgttccaaaagagaaacaccggaacatgctaca
6801 ---------+---------+---------+---------+---------+---------+ 6850
     L  A  F  P  G  S  V  P  K  E  K  H  R  N  M  L  Q  -

     ataccagcttccatcttcattatcttctctggccaggatattcagcatcc
6851 ---------+---------+---------+---------+---------+---------+ 6900
      Y  Q  L  P  S  S  L  S  S  L  A  R  I  F  S  I  L -

     tctcccagagcaaaaagcgactccacatagaagactactctgtttctcag
6901 ---------+---------+---------+---------+---------+---------+ 6950
       S  Q  S  K  K  R  L  H  I  E  D  Y  S  V  S  Q  -

     acaacacttgaccaagtatttgtgaactttgccaaggaccaaagtgatga
6951 ---------+---------+---------+---------+---------+---------+ 7000
     T  T  L  D  Q  V  F  V  N  F  A  K  D  Q  S  D  D  -

     tgaccacttaaaagacctctcattacacaaaaaccagacagtagtggacg
7001 ---------+---------+---------+---------+---------+---------+ 7050
      D  H  L  K  D  L  S  L  H  K  N  Q  T  V  V  D  V -

     ttgcagttctcacatcttttctacaggatgagaaagtgaaagaaagctat
7051 ---------+---------+---------+---------+---------+---------+ 7100
       A  V  L  T  S  F  L  Q  D  E  K  V  K  E  S  Y  -

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7101 ---------+---------+---------+---------+---------+---------+ 7150
     V  *

     actttcctttgcaccatgtgaagtgttgtggagaaaagagccagaagttg
7151 ---------+---------+---------+---------+---------+---------+ 7200


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7201 ---------+---------+---------+---------+---------+---------+ 7250


     caattcaatg
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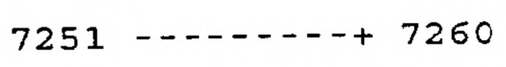
7251
7260

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 00 10 5820

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,X	BODZIOCH M ET AL: "THE GENE ENCODING ATP-BINDING CASSETTE TRANSPORTER 1 IS MUTATED IN TANGIER DISEASE" NATURE GENETICS,NEW YORK, NY,US, vol. 22, no. 4, August 1999 (1999-08), pages 347-351, XP000889766 ISSN: 1061-4036 * page 349, column 2, line 3 * ---	1-11,14, 15	C12N15/12 C12N15/11 C12N9/00 C12N5/10 C07K14/705 C07K16/28 C12Q1/68 G01N33/53 A61K48/00
D,X	BROOKS-WILSON A ET AL: "MUTATIONS IN ABC1 IN TANGIER DISEASE AND FAMILIAL HIGH-DENSITY LIPOPROTEIN DEFICIENCY" NATURE GENETICS,NEW YORK, NY,US, vol. 22, no. 4, August 1999 (1999-08), pages 336-345, XP000889767 ISSN: 1061-4036 * figures 7,8 * ---	8-10	

-/--

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12N
C07K
C12Q
G01N
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely

Claims searched incompletely

Claims not searched

Reason for the limitation of the search

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	5 December 2000	Lonnoy, O

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH
SHEET C

Application Number
EP 00 10 5820

Claim(s) not searched:
12,13

Reason for the limitation of the search:

Present claims 12 and 13 relate to a compound defined by reference to a desirable characteristic or property, namely that it is a modulator of polypeptides of the invention. The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for no such compound. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search can be carried out for such claims, the wording of which is a mere recitation of the results to be achieved.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 00 10 5820

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D,X	LAWN R M ET AL: "THE TANGIER DISEASE GENE PRODUCT ABC1 CONTROLS THE CELLULAR APOLIPOPROTEIN-MEDIATED LIPID REMOVAL PATHWAY" JOURNAL OF CLINICAL INVESTIGATION,NEW YORK, NY,US, vol. 104, no. 8, October 1999 (1999-10), pages R25-R31, XP000884782 ISSN: 0021-9738 * figures 2-5 *	8-10	
E	WO 00 18912 A (BAYER AG ;KLUCKEN JOCHEN (DE); SCHMITZ GERD (DE)) 6 April 2000 (2000-04-06) SeqIdNo.1: 100.0% identity in 6880 bp overlap with SeqIdNo.1 / SeqIdNo.2: 100.0% identity in 2201 aa overlap with SeqIdNo.2	8-10	
A	YOUNG S G ET AL: "THE ABCS OF CHOLESTEROL EFFLUX" NATURE GENETICS,US,NEW YORK, NY, vol. 22, no. 4, August 1999 (1999-08), pages 316-318, XP000889764 ISSN: 1061-4036		TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

European Patent Office

Application Number
EP 00 10 5820

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☒ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 00 10 5820

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-15 (all partially)

   G596A allele of ABC1, recombinant methods, products, therapeutic, and diagnostic applications relating thereto.

2. Claims: 1-15 (all partially)

   T1136C allele of ABC1, recombinant methods, products, therapeutic, and diagnostic applications relating thereto.

3. Claims: 1-15 (all partially)

   A2589G allele of ABC1, recombinant methods, products, therapeutic, and diagnostic applications relating thereto.

4. Claims: 1-15 (all partially)

   G3456C allele of ABC1, recombinant methods, products, therapeutic, and diagnostic applications relating thereto.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 10 5820

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-12-2000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0018912 A	06-04-2000	AU 5980499 A	17-04-2000

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82